ANONYMOUS HIV INCIDENCE STUDY IN ROUTINE AUTOPSY CASES

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BONAFIDE CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"ANONYMOUS HIV INCIDENCE STUDY IN ROUTINE AUTOPSY CASES"** has been carried out by **Dr. R. SANGEETHA, M.B.B.S.,** a Post Graduate student, under my supervision and guidance for her study leading to Branch XIV M.D. Degree in Forensic Medicine during the period from May 2010 to April 2013

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DECLARATION

I, Dr. R. Sangeetha., solemnly declare that this dissertation titled **"ANONYMOUS HIV INCIDENCE STUDY IN ROUTINE AUTOPSY CASES"** is the bonafide work done by me under the expert guidance and supervision of **Capt. Dr. B. Santhakumar M.Sc., MD., DipNB (FM), P.G.D.M.L.E**, Director and Professor, Institute of Forensic Medicine, Madras Medical College, Chennai – 3. This dissertation is submitted to The Tamil Nadu Dr. M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch XIV) in Forensic Medicine.

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ABBREVIATIONS

AIDS – Acquired Immune Deficiency Syndrome

CAEV – Caprine Arthritis Encephalitis Virus

CDC – Centres for Disease Control and Prevention

CCR – Chemokine Co-Receptor

CMV – Cytomegalovirus

CRF – Circulating Recombinant Forms

DNA – Deoxy-ribo Nucleic Acid

EBV – Epstein Barr Virus

ELISA – Enzyme Linked Immunosorbant Assay

FIV – Feline Immunodeficiency Virus

GRID – Gay Related Immune Deficiency

HAART – Highly Active Antiretroviral Therapy

HBV – Hepatitis B Virus

HCV – Hepatitis C virus

HIV – Human Immunodeficiency Virus

HTLV – Human T- Lymphotropic Virus

KS – Kaposi Sarcoma

LAV – Lymphadenopathy Associated Virus

NAT – Nucleic Acid Amplification Test

NNRTI – Non- Nucleoside Reverse Transcriptase Inhibitor

NRTI – Nucleoside Reverse Transcriptase Inhibitor

PCP – Pneumocystis Cariini Pneumonia

PCR – Polymerase Chain Reaction

PEP – Post Exposure Prophylaxis

PGL – Persistent Generalized Lymphadenopathy

PI – Protease Inhibitor

RIBA – Recombinant Immunoblot Assay

RNA – Ribonucleic Acid

SIV – Simian Immunodeficiency Virus

WB – Western Blot

WHO – World Health Organization

ABSTRACT

Acquired Immunodeficiency Syndrome (AIDS) caused by Human Immunodeficiency Virus (HIV). The postmortem examination room is the place where infection can spread to persons who are performing autopsy. These personnels are having high risk of various injuries, infections. Forensic personnels who are involved in examination of homosexuals and drug abusers, are having great chance of being infected by HIV than common people. We conducted the Study to find the incidence of HIV in routine autopsy cases.

Materials and methods: The study sample consists of 486 routine autopsy cases at Rajiv Gandhi Government General Hospital. The samples were tested using an enzyme immunoassay using SD Bioline HIV1/2 3.0 Rapid test kits to detect the presence of HIV-1 and HIV-2 antibodies. Samples yielding reactive results were confirmed by Alere Determine^{TM} HIV 1/2 method which detects HIV-1, HIV-2 antibodies.

Conclusion: Out of 486 subjects in this study group, 4 cases were positive for HIV and all were previously unknown seropositive cases. This shows that HIV screening is of great importance among the community where the crime rate is high. Homicide victims in our study showed a relatively higher prevalence of HIV-1, infections compared with other manners of deaths.

INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) caused by Human Immunodeficiency Virus (HIV). HIV is a disorder of the immune system in which there is a breakdown of defense mechanism against infection, which leaves the person susceptible to the life-threatening infectious diseases including unusual malignancies.[1]

It is estimated that about 14,000 HIV infections occur every day around the world approximately and out of these, 90% of these are in developing countries[2]. HIV is of two types namely HIV-1 and HIV-2, both of these viruses can cause manifestations, which cannot be distinguished from each other. The difference remains in the onset of the disease; HIV-2 has a delay in onset of disease[3]. Universally, infections rates are maximum in sub-Saharan Africa and are also are high in southeast Asia.[4]

The postmortem examination room is the place where infection can spread to persons who are performing autopsy. These personnels are having high risk of various injuries, infections.[5,6] Forensic personnels who are involved in examination of homosexuals and drug abusers, are having great chance of being infected by HIV than common people. Forensic experts are exposed not only to scalpels, needles but also to the bones, body fluids and tissues of dead bodies every day[8].

Autopsy safety was not of much attention till 1980, it was taken in to consideration only when HIV infection appeared[8]. Testing for HIV in the autopsy room has not been done routinely because samples to be tested need a special equipped laboratory, well trained technician and also more time is needed to obtain the results. Therefore a simple, reliable and rapid means for detecting the antibodies to HIV infection is needed and this can be useful for screening in the autopsy rooms[9]. Since there are no clear strategies as when a deceased body should no longer be considered infectious.[9]

Only limited data is available regarding the occupational transmission of the HIV from the corpses to the persons who engaged directly or indirectly in the autopsy procedures and other individuals who handle the dead bodies of various stages of decomposition[10]. The purpose of this study is to determine whether autopsies of the corpses, which have been presumed to be low risk groups, are safe or not. Thus, we conducted a study to identify the seroprevalence of these viruses in a low risk forensic autopsy population.

Testing HIV antibodies during autopsies has been useful not only for assessing the risk to the mortuary workers but also useful for the epidemiological studies[11]. Many simple, rapid and reliable tests for detecting the antibodies are needed for reducing occupational health

hazards in the mortuary, because the HIV may survive for several days in the postmortem samples. The applicability of the tests for detecting antibodies to HIV in autopsy samples depends on the postmortem stability of the antibodies as well as on the sensitivity of the screening tests[12].

In a population, where there is a low HIV prevalence, refusals from screening for HIV tests by the people with risk behavior may occur and this strongly bias the seroprevalence. The social habits, human ethical issues and religious customs becomes an obstacle to obtain the information about the dead bodies[13,14].

Autopsy screening efficiently reveals the true incidence in groups where refusal may be common. This study enables to identify HIV status of the deceased whose HIV status was unknown previously because it is not practical or cost-effective to take full universal precaution with every autopsy.[14]

AIMS AND OBJECTIVES

1. To Study the incidence of HIV in routine autopsy cases.
2. To create awareness among the doctors doing postmortem and the mortuary workers.
3. To diagnose the clinically undetected HIV cases.
4. To determine whether postmortem of deadbodies which are thought to be at low risk groups, are safe or not.
5. To formulate guidelines for a policy concerning HIV antibody screening in forensic autopsy cases
6. To determine how much autopsies are safe in relation to pre-autopsy testing for HIV.
7. To identify the seroprevalence of HIV in autopsy room personnels.

REVIEW OF LITERATURE

The situation of Forensic Pathologists to perform autopsies in cases whose HIV status is not known prior to autopsy. A person infected with HIV may die of an unrelated cause, in those individuals there is no clear-cut evidence of HIV status. HIV incidence is the measure of new cases in a given period of time. This occurrence of new cases helps us to supervise HIV epidemic in the country. This information also helps us to create or modify the awareness programme to educate the general population and high risk community who are vulnerable to HIV infection.

HIV antibodies testing in autopsies have been valuable for epidemiological purposes[15, 16]. Consistent tests may also be needed to reduce the occupational health hazards in the mortuary, because the HIV may survive for several days in postmortem samples[17].

The applicability of the tests for detecting HIV antibodies in autopsy samples depends on the postmortem stability of the antibodies as well as on the sensitivity of the screening test. The globulins that contain antibodies are not affected by postmortem autolysis, or bacterial contamination [18].

Medico legal autopsies include fatal traffic accidents and sudden death, which is considered to provide the most representative sample of

the living population. Moreover, homosexuals and drug addicts with a high HIV-infection risk often commit suicide or otherwise die violently and are thus subjected to medico legal autopsy[19]. Hence, HIV-testing in medico legal autopsies would be a valuable method in searching for 'hidden' HIV carriers [20, 21].

When the forensic pathologists conduct postmortem on dead-bodies that are in various stages of decomposition, a thorough autopsy of the soft tissue and skeleton is necessary to come to a conclusion regarding the identity and cause of death especially in suspicious cases[22]. Irrespective of the stage of decomposition, the chance of exposure to various infectious organisms in the body fluids and tissues is very high. An exposure to HCW (Health care worker) at risk of HIV is defined as a needle-stick or cut in the connective tissue by sharp instruments, exposure to mucosa or contact with intact or cut skin when the period of exposure is extended[23].

The studies conducted prospectively have assessed that approximate risk of infection by HIV after a percutaneous needle injury is at a rate of 0.3%[24]. In the context of an autopsy it is important that not only blood but also other body fluids that are highly infectious like amniotic fluid, pleural fluid, seminal fluid, pericardial fluid, peritoneal fluid, vaginal fluid and cerebrospinal fluid.

Weston and Lober et al. have recognized that rupture of the surgical glove occurs in about 9% of autopsy. About 33% of glove puncture goes undetected by the pathology personnels, which can expose the previous injury in the hand to come in contact with infected blood for a long time[25].

Coleman, D.L. Luce et al (1986)[26] conducted a study to detect antibodies to the Retrovirus in presumably healthy San Franciscans who died unexpectedly. Of the 121 samples tested for antibodies to HIV, 23 cases were positive for HIV which was very high than other voluntary screening programs, which shows that abstaining the high risk individual from testing may cause serious errors in all kinds of voluntary surveillance programs.

Schoub, B. D., Johnson, et al (1989)[27] conducted a study to detect the HIV seropositivity in sera from medico legal autopsies in Johannesburg, out of the 745 samples tested 6 (0.8%) were positive for HIV antibodies.

Dupon, M., Bonnici, et al (1989)[28] in his study Screening for HIV in Necropsies, of the 114 samples tested for HIV antibodies in the city of Bordeaux 5(4.6%) samples were reactive.

Klatt et al (1989) assessed the reliability of postmortem enzyme immunoassay testing for antibodies to HIV and determined that vitreous humor is reliable for testing antibodies to HIV up to 34 hours postmortem and blood atleast up to 58 days were frequently positive for HIV. He concluded that Postmortem enzyme immunoassay testing of vitreous humor blood and may be valuable to screen for screening HIV infection in high-risk groups.[40]

Little and Ferris et al (1990)[29] Tested for the presence of antibodies to HIV in Forensic autopsies at Vancouver, using the recombinant immunoblot assay (RIBA) technique. 207 forensic autopsy cases were tested for the HIV antibodies. Out of which, 172 persons were without any known history of HIV. In this only 2 cases were positive for HIV, but the results were not confirmed by further testing. The RIBA HIV test system has 100% sensitivity and 98.5% specificity. However this procedure is time consuming and this requires special equipment so it is not suitable for routine diagnosis for autopsy screening.

Karhunen, et al (1992)[14] Detected the HIV antibodies in medico legal autopsies using an enzyme immunoassay on postmortem sera was conducted for a period of 5 years. This study consist medicolegal autopsies of all deaths under the age of 65 years and the study sample was 7305. Out of which nine (0.12%) were positive for HIV. 7 of these cases

were previously known case of HIV and remaining 2 were unknown. He concluded that testing of HIV in medico legal autopsies helps in identifying the new cases of HIV. These HIV testing have no ethical issues and may be sensitive to early changes in epidemiology.[14]

David Sadler et al (1992)[30] studied the prevalence of HIV antibodies using ELISA in the 500 postmortem samples to detect the antibodies to both HIV1 and HIV2, the positive results were confirmed by second ELISA. He classified the individual in to risk and no known risk individual based on the history from police, practitioner and from hospital records. He concluded that autopsy population contains high risk individual, therefore testing for HIV antibodies in these individuals is useful epidemiologically.

Kringsholm, et al (1993)[31] Studied the incidence of HIV-1 antibodies among 389 individuals who are addicted to drugs. The study was conducted in a medical institution at Copenhagen. The incidence of HIV 1 was almost equal in both male and female. In around one third of the HIV-1-positive cases, the HIV status was not known previously. Therefore he concluded that in autopsied drug addicts the diagnosis of HIV-1-infection is significant for epidemiological data and also for safety measures.

Li, Zhang, Constantine et al (1993)[32] Studied the Seroprevalence of HBV, HCV, HIV-1 in forensic autopsy, using ELISA to analyze the risk factors connected with it. A total of 414 serum samples were tested for HBV, HCV, HIV-1, HTLV-I and HTLV-II antibodies in autopsy cases successively. Of the 414 cases, about 6% were positive for HIV-1, 19.1% for HCV, 23.2% for HBV, and 1.0% for HTLV-I and HTLV-II. He established that the overall HIV 1 prevalence was greater than the general population at Maryland. Routine testing only for HIV-1 may miss other infections like hepatitis C virus or hepatitis B virus. This study recommends universal precautions for all autopsy cases.

Lockemann et al (1993)[33] studied the HIV-status of 3999 autopsy cases from 1989-1992 at Hamburg. All the autopsy cases included in the study were with unnatural death and the cause of death was not known. Primarily HIV was tested by Enzyme Linked Immuno Sorbant Assay and confirmed by Western-blot. Confirmation was done in 55 positive cases using Western-blot. The seroprevalence of HIV was 1.4%. He concluded that Serological HIV-testing of drug associated deaths helps in epidemiology screening among high-risk group.

Douceron and Deforges et al (1993)[34] conducted the study to carry out the postmortem safely without the risk of infection by HIV. In this study, they cultured the blood serially for a variable postmortem

interval. Viable HIV was isolated from the blood sample on 17th day of autopsy, pleural fluid on 14th day of autopsy, and pericardial fluid on 16th day of autopsy. He concluded that there is no time period when autopsy can be performed without risk of infection to HIV. Therefore delay in performing postmortem will not abolish the risk of HIV infection.

Henrikki Brummer et al (1994)[35] studied the stability of antibodies to HIV from serum samples and bilious fluid collected from 8 HIV-positive postmortem cases. The postmortem interval of the autopsy was on an average of 5 days. Serum and bilious fluid samples were stored at 27°C for 50 to 310 days for testing. Detecting antibodies to HIV in postmortem samples depends on the stability of the antibodies and sensitivity of the screening tests. He concluded that HIV antibodies can be detected for weeks to months in postmortem specimens, even if stored at room temperature. Postmortem testing for antibodies to HIV in autopsies thus appears to be a reliable for monitoring the prevalence of asymptomatic carriers in autopsy series and screening for safety purposes.

Zehner et al (**1995**)[36] conducted the study to detect HIV antibodies in postmortem blood samples. Before commencing the autopsy, totally 456 samples were collected and was tested using the HIV 1/HIV 2 Test pack. The samples showing positive result for HIV were confirmed by Western blot. Among the study sample, 21 cases were proved to be

positive and it was confirmed by Western blot. The study concludes that this HIV-Test pack gives proper results to HIV antibodies in whole blood.

Cattaneo et al (1999)[37] studied the Prevalence of antibodies to HIV among the blood samples from the medico legal cases. This study was conducted in 397 individuals aged between 16 to 50 years. These cases were tested for HCV and HIV antibodies. Out of 134 individuals who were tested positive overall, 20 individuals were found positive for HIV antibodies alone, 69 individuals were found positive for HCV antibodies alone, and 45 individuals were found positive for both HIV and HCV antibodies. Cattaneo et al concluded that significant population had risk of dangerous infection in medico legal autopsies.

Tjotta et al[38] demonstrated that HIV survived in dried blood at room temperature for up to five or six days if the optimum pH level is maintained. The HIV infectivity in the blood does not appear to be affected by drying.

Hossein Sanaei-Zadeh (2002)[39] **s**tudied the seroprevalence of HBV, HCV and HIV in autopsies, at Tehran. Postmortem blood samples were collected in 173 cases for a period of 1 year, out of which 8 were positive for Hbs Ag, and 7 cases were also positive for Hbs Ag and anti-HCV. No case was positive for anti-HIV 1 and HIV 2. He concluded that all medico legal autopsy cases, including cases that were thought to be at

low risk, must be considered as highly and dangerously infectious. Therefore suitable precaution must be carried out during necropsies.

Kaplan JC, Allan JD, Groopman JE et al[40] isolated the viable HIV from cranial bone, cerebrospinal fluid and brain up to five days after death.

Nyberg et al[41] Studied the viability of HIV in post mortem samples and concluded that HIV-infected patients should apparently be considered potentially infectious for at least one or two weeks postmortem. HIV was cultured from tissue specimens at autopsy up to 6 days post mortem and from spleen specimens stored for 14 days. He suggested precautions, including for bone, during autopsy of HIV-infected patients even after extended postmortem intervals.

Johnson et al[42] documented occupational transmission of HIV to a forensic pathologist in the US who sustained injury with scalpel.

Eriksen MB et al (2009)[43] reported the Postmortem Detection of HBV, HCV and HIV genomes in the blood samples among deaths in drug addicts in Denmark with rapid kits. He collected the blood samples from autopsy cases and screened for HIV antibodies and hepatitis antibodies. The screening of viral genome was performed by polymerase chain reaction (PCR) technique. HIV genome was detected positive in about

40% of samples. Hepatitis B virus genome was detected in about 20% of anti-HBc – positive/anti-HBs-negative samples.

Sonia Mehta et al (2012)[8] conducted Pre-testing Screening for HIV antibodies before Conducting Post-mortem. A total of 328 samples of blood were collected prior to the autopsy and tested for antibodies to HIV for a period of three years. The study was routine confidential and anonymous testing for HIV antibody. Only 2 samples (0.6%) were found to be HIV reactive, average postmortem interval was about 24 hours. None of the cases had a known HIV status. This study concluded that universal precautions as suggested in autopsy cases not practically possible in a developing country like ours, therefore such pretesting may be necessary.

HISTORY AND DISCOVERY OF AIDS

DISCOVERY OF HIV:

AIDS was first recognized in the year 1981[44], with reports of a sudden unexplained outbreak of two very rare diseases, Pneumocystis cariini pneumonia (PCP), and Kaposi sarcoma (KS). It is usually seen among drug addicts and homosexuals. They lost their immunity and becoming more prone to many opportunistic infections and other secondary neoplasms. Many cases of PCP and KS emerged in the year 1981 and it alerts the CDC and this made the CDC to observe the outbreak[45].

Initially, the CDC has not coined official name for AIDS. It was in the year 1981, the term GRID was used by the general press, which stands for gay related immune deficiency [46]. Later the CDC had coined the name as "*the 4H disease*" as it mean for Haitans, Homosexuals, Haemophiliacs and Heroin users[47]. Later it was determined that AIDS was not only related to the gay community alone. It was recognized that the name GRID was deceptive, therefore the name AIDS in the year 1982. Then CDC uses the name Acquired immune deficiency syndrome.[48]

In 1983, Robert Gallo in United States and the French scientist Luc Montagnier from the Pasteur Institute of Paris independently stated that a retrovirus is the cause of AIDS. Gallo claimed that a virus isolated from an AIDS patient was similar to other (HTLVs). Robert Gallo gave the name for this newly isolated virus as HTLV-III[49].

Montagnier's et al isolated a virus from a individual presenting with, classic symptoms of AIDS. Montagnier revealed that core proteins of the isolated virus were immunologically different from those of HTLV-I and named that isolated virus as lymphadenopathy-associated virus (LAV)[45]. During the commencement of 20th century HIV appeared first in humans in Africa as a result of SIV infection.

Serological tests were available for the anti-HIV antibodies detection. The estimation of the extent of HIV infection is made more realistic only after the serological tests available. The generic name human deficiency virus (HIV) [3] was finalized in 1986 by international committee on Virus Nomenclature.

HIV-1 and HIV-2 are the result of several cross-species transmissions of simian immunodeficiency viruses (SIVs) naturally infecting African primates. Both HIV-1 and HIV-2 seemed to have originated in non-human primates in West-central Africa and by a process

known as zoonosis; it was transferred to humans in the early 20th century[50].

The origin of HIV-1 is through the evolution of SIV, a simian immunodeficiency virus in southern cameroon that infects chimpanzees[51, 52]. SIV is the closest relative of HIV-2, a virus of the sooty mangabey residing in West Africa[53]. HIV-1 is believed to have skipped the species barrier on three distinct occasions, giving rise to the three groups of the virus namely, M, N, and O[53].

It is believed that human beings who take part in chopping meat of monkeys, either as vendors or hunters, usually get SIV[54]. Simian immunodeficiency virus needs several transmissions from one person to another person in rapid succession to give sufficient time for mutation to HIV[55].

The earliest well recognized case of HIV in human being dates back to 1959 in the Congo[56]. It have been existing in the United States as early in 1966[57], but the infections outside the Africa can be found back to a single person who got infected with HIV in Haiti and then transported the infection to the United States around[57].

Among HIV-1 and HIV-2, HIV-1 is closely related to virus that was isolated from species of chimpanzees and gorillas. The chimpanzee

sub-species *Pantroglodytes* have been established to be the natural reservoir of the HIV-1 M (Major) and N (New) groups. The HIV-1 O (Outlier) group is related to the viruses that originate in Cameroonian gorillas[58].

The M group consists of nine subtypes, named as A, B, C, D, F, G, H, J, and K and circulating recombinant forms (CRFs) [59]. By infection of an individual with two different subtypes to create a new virus with a selective advantage is known as circulating recombinant forms CRFs .

STRUCTURE OF HIV:

HIV belongs to a retrovirus class of viruses. Within this class, it comes under the subgroup called lentivirus. Some other lentiviruses include Simian Immunodeficiency Virus (SIV), Feline Immunodeficiency Virus (FIV), Visna and (CAEV) Caprine Arthritis Encephalitis Virus.

Mostly viruses store their genome as long strands of DNA but Retroviruses are composed of RNA. HIV is an enveloped, spherical, icosohedral virus about 90-120 nm in size. The outer envelope consists of lipid bilayer with regularly arranged spikes or knobs which are 72 in number. The outer envelope of HIV-1 has gp120/gp140, whereas the outer envelope of HIV2 has gp41/gp36.Both are responsible for HIV infection.

The virus core contains

(1) capsid protein p24;

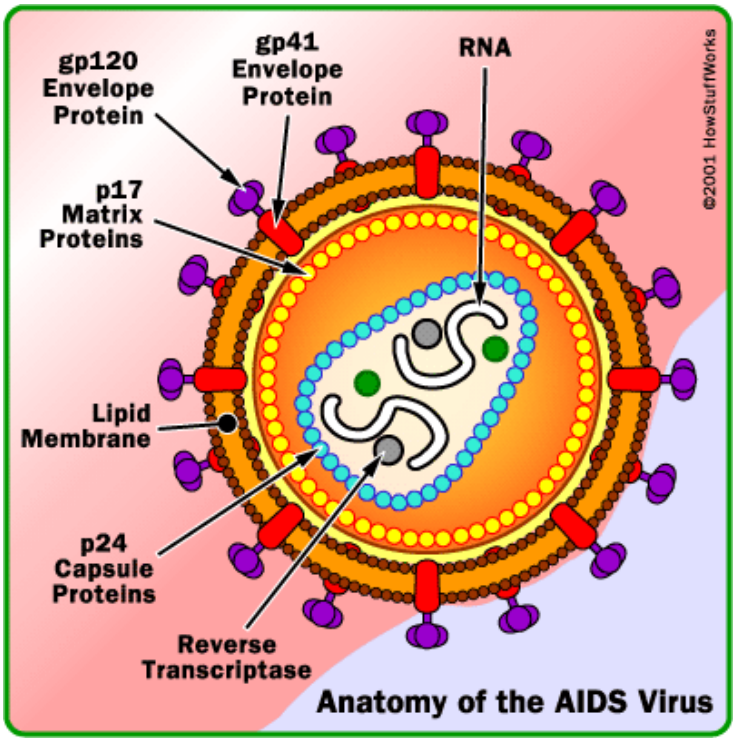
(2) nucleocapsid protein p7 / p9-Regulation of gene expression

(3) 2 copies of genomic RNA; and

(4) 3 enzymes (protease, reverse transcriptase, and integrase) for viral replication and maturation.

The viral antigen p24 is used for the diagnosis of HIV infection in the Enzyme-linked immunosorbent assay (ELISA). The outer core of the

viral is bounded by a matrix protein called p17, which lies underneath the envelope.



The diploid genome composed of 2 identical single-stranded, positive sense RNA copies. When the virus infect the cell ,viral RNA is transcribed by the enzyme reverse transcriptase , into single stranded DNA and then to double stranded DNA (Pro virus) which is integrated into the host cell. This provirus remains latent for long period and in response to viral promoters it commences the viral replication by the synthesis of viral RNA.

The HIV-1 RNA genome contains the gag, pol, and env genes, which are typical for retroviruses. The gag and pol genes products are

translated primarily into large precursor proteins and then cleaved by the viral enzyme protease to produce the fully matured proteins. HIV comprises numerous accessory genes such as *vif*, *nef*, *tat*, *rev*, *vpr*, and *vpu*, which control the synthesis and assembly of infectious viral particles and its pathogenicity.

TYPES:

HIV is a highly inconsistent virus which readily mutates according to the necessity. There are different strains of HIV in different individuals and many strains of HIV can be seen within the body of the same individual. HIV can be classified in to two types genetically and antigenically. On the basis of genetic similarity, HIV strains can be classified further into groups and subtypes.

HIV-1 is worldwide predominant while HIV-2 is found mainly in West Africa but rarely elsewhere. Both types are transmitted in the same way, and they cause clinically indistinguishable AIDS. The transmission of HIV-2 is less compared to HIV-1, and the incubation period which is between the time of infection to appearance of symptoms of illness for HIV-2 is longer. HIV-2 is closely linked to the SIV found in West Africa.

SUB-GROUPS AND SUB-TYPES:

The HIV-1 strains can be categorized into four Subgroups: the major group M, the outlier group O and two new groups, N the new and another strain P. The strain P was found in 2009 closely linked to gorilla simian immunodeficiency virus.

Group M constitutes greater than 90 percent of HIV-1 infections and are responsible for world pandemic. In group M there are nine genetically distinct subtypes. Based on nucleotide sequence analyses of the *env* and *gag* genes, within the M group of HIV-1 there are also at least 10 different subtypes they are designated from A to K. [60]

Type C is the predominant form not only in India but caused the most infections worldwide. The Subtype B is most prevalent in the Americas and Europe, but globally subtypes C accounts for half of all strains[62]. The reason for high prevalence of subtype C is due to the predominance of it in Africa and India. Subtype D is more virulent due to its effective binding to immune cells[61]. The Type O HIV-1 is often found in Cameroon and Gabon and the rare sub-group N is also found in Cameroon.

HIV-2 infection differs from HIV-1 in being inherently resistant to non-nucleoside reverse transcriptase inhibitors (NNRTIs) and patients

have low viral load, lower rates of vertical transmission, slower decline in CD4 count and several fold slower progression to AIDS. Rarely, many subtypes can meet in the cell of an infected person and combine their genetic material to generate a "circulating recombinant forms" or CRFs is a mixture of different subtypes**.**

MODES OF TRANSMISSION:

HIV infection is a communicable disease and can be transmitted from person to person, most commonly by having unprotected sexual intercourse. HIV is present in blood, semen and other body fluids such as breast milk, urine, saliva, sweat, tears, pre-ejaculated fluid and vaginal fluid and can be transmitted by various methods. Worldwide, heterosexual transmission being the major route (>75%) and vertical transmission (5-10%)[63].

SEXUAL CONTACT:

HIV is a sexually transmitted disease (STD) and it seems to be transmitted from female to male with greater effectiveness than from male to female. It can be transmitted through unprotected sexual act from an infected individual. The risk of becoming infected with an act of unprotected sexual practice depends on the amount of virus in the body fluids, the presence of other sexually transmitted diseases.

VERTICAL TRANSMISSION:

HIV positive women can transmit HIV to her child either during pregnancy or during the process of delivery or by the route of mother's milk. The HIV infected pregnant woman can transmit to fetus or newborn a rate varies from 15-40%. This rate can be reduced to levels below 5%

with effective interventions. HIV is thought to be transmitted during the last weeks of pregnancy or during delivery[63].

OCCUPATIONAL EXPOSURE:

An occupational exposure may place a worker at risk of HIV infection through injuries such as those involving a potentially contaminated needle or sharp instrument or chapped, abraded skin or contact with mucous membranes. The risks for occupational transmission of HIV differ with the type and severity of exposure. Seroconversion is defined as a situation in which seronegative health care worker sustains an injury with a device soiled with blood or body fluids from a HIV seropositive or unknown source and seroconverts within the succeeding 6 months.

The average risk for HIV transmission after percutaneous exposure to HIV-infected blood has been expected to be about 0.23%[64]. Average risk after a mucous membrane exposure is estimated to be approximately 0.09% Factors associated with an increased risk of transmission comprises: deep injury, injury produced by a device that penetrates a blood vessel, hollow-bore needle injury, and a source with high viral load[8].

NEEDLE SHARING:

Injectable-drug users share the same needles where the blood contaminated needle of one person come into direct contact with other individual. Sharing contaminated needles with individuals infected by HIV will infect the new individual, due to the direct contact of blood of infected individual. Accidental needle prick injuries are common in health care workers but the risk of HIV transmission through needle prick injury is less than 1 percent.

DIFFERENCES IN TRANSMISSION AMONG SUBTYPES:

Specific subtypes or CRFs of HIV are specifically related to specific mode of transmission[1]. The Subtype C and CRF A/E are spread mostly by heterosexual contact, while subtype B is spread mostly by homosexual contact and drug addiction.

Percentage of risk in different modes:

- Sexually – about 0.5%
- Parenterally – about 90%
- Vertical transmission – 15-40 %
- Drug addiction - 0.5-1.0%
- Occupational transmission - 0.2%

PATHOPHYSIOLOGY OF HIV:

After the entry of virus inside the human body there is a rapid replication of virus resulting in a great quantity of virus in the peripheral blood. There are several million virus particles per milliliter of blood during primary infection. It is accompanied by reduction in the circulating $CD4^+$ T cells. This is followed by the activation of CD8 T cells, which kill HIV-infected cells, and subsequently produces the antibody, known as sero conversion period. When the $CD8^+$ T cell response is good, the disease progression is slowed down and a better prognosis is obtained.

HIV causes AIDS by depleting $CD4^+$ T helper lymphocytes which weakens the defense mechanism and makes the individual susceptible to opportunistic infection.

In the acute phase HIV induces lysis of cells and killing of infected cells by activation of cytotoxic T cells occurs which accounts for depletion of CD4 T cell. In the chronic phase, the generalized activation of defense mechanism with the gradual loss of the capacity of the body to generate new T cells results in slow decline in $CD4^+$ T cell numbers.

The characteristic symptoms of AIDS does not appear for many years after a person is infected, the loss of $CD4^+$ T cell occurs during the

first few weeks of infection, particularly in the mucosa of the intestine, which harbors most of lymphocytes present in body. HIV lodges and abolishes CCR5 expressing $CD4^+$ T cells during acute infection.

NATURAL HISTORY OF HIV/AIDS:

HIV infection has 4 stages:

1) Primary infection

2) Early immune deficiency

3) Intermediate immune deficiency

4) Advance immune deficiency

1) Primary infection

There is a fast proliferation of the virus in the lymph nodes and blood following HIV infection. In this stage there is a sero conversion illness which resolves within weeks. There is a rapid declination of the CD4 count before the virus is controlled by the immune system.

2) Early immune deficiency

In this stage, the immune system has controlled the virus particularly the lymphoid tissue and people who are infected with HIV are usually asymptomatic.

3) Intermediate immune deficiency

There is a rapid replication of virus and CD4cell turn over and CD4 cell count will be around 200-500 count/microlitre, Signs and symptoms begin to appear in this stage.

4) Advance immune deficiency

The virus proliferates all over the body and overcomes the defense system. Oppurtunistic infections and chance of malignancies are common in this stage.

CLINICAL FEATURES OF HIV INFECTION:

The WHO system uses the following classifications:

STAGE 1 : Primary infection,

STAGE 2 : Clinically asymptomatic stage,

STAGE 3: AIDS-related complex or persistent generalized lymphadenopathy,

STAGE 4 : AIDS.

STAGE 1: Primary HIV infection

This primary HIV infection lasts for a few weeks and is frequently accompanied by a flu-like illness for a short period. This is seen in 10% of individuals and matches with seroconversion. They present with mononucleosis like illness which includes sore throat, fever, lymph node enlargement, joint pain, skin rash, and malaise.

In this stage, the defense mechanism of the body responds to the virus by producing antibodies to HIV a process called seroconversion. Large amount of virus will be present in the peripheral blood in this primary stage.

STAGE 2: Clinically asymptomatic stage

As the name indicates, this stage is without major symptoms, and it lasts approximately for ten years. In the peripheral blood, the HIV falls to low levels but still the people remain infectious. Antibodies to HIV may be detected in blood, showing positive result. Children will have a shorter incubation period. Only lymph node enlargement may be present in this stage.

STAGE 3: AIDS-related complex

In this stage the immune system becomes impaired severely. This is due to:

- Impairment of the lymph nodes.
- HIV mutation, leading to T helper cell impairment.
- Failure to replace T helper cells that are lost.

This stage is usually characterized by multi-system disorder which comprises of immunology, dermatology, hematology and nervous system. Constitutional symptoms, such as weight loss, fever and night sweats, may develop. Generalized lymph node enlargement may be present.

STAGE 4: AIDS

The period of clinical latency differs in time from 1 to 2 years to more than 15 years .The transition from stage 3 to full-blown AIDS may happen quickly or slowly. The progression of the disease is predisposed by stress, cofactors, and genetic factors. The poor prognostic factors include decrease in the CD4 lymphocyte count and the reappearance of HIV antigen in the blood. The individual may develop gradually severe opportunistic infections as the immune system becomes severely damaged, leading eventually to a full blown disease. In this stage, opportunistic infections such as protozoa, fungi, bacterial and viral diseases are seen in patients with AIDS.[68]

OPPORTUNISTIC INFECTIONS

PROTOZOAL

Toxoplasmosis

Pneumocystis Carinii

Cryptosporidiosis

BACTERIAL

Mycobacterium avium complex

Atypical mycobacterial disease

Extra-pulmonary TB

Salmonella septicemia

Multiple or recurrent pyogenic bacterial infection

FUNGAL

Candidiasis

Crytococcosis

Histoplasmosis

Coccidiodomycosis

VIRAL

Cytomegalovirus

Herpes Simplex Virus

Varicella Zoster Virus

Opportunistic infection is caused by bacteria, protozoa, fungi, and viruses in patients with AIDS. Pneumocystis carinii causes pneumonia is the most common opportunistic infection in patient with AIDS. The classic symptoms are breathlessness, cough and fever. Diagnosis includes bronchoscopy and the detection of Pneumocystis in the lavage. Pentamidine and co-trimoxazole are the treatment of choice.

Toxoplasmosis is dangerous because it affects brain. The treatment is a pyrimethamine and sulfonamide combination. Cryptosporidium often causes persistent diarrhea.

The second most common opportunistic infection in a patient with AIDS is candida albicans. Oesophageal candidiasis is most commonly seen in patients with pre-AIDS. Treatment is with amphotericin or ketaconazole. Cryptococcus neoformans infection is fatal, if untreated. Diagnosis may be made earlier by the demonstration of the fungal antigen or fungus in the cerebrospinal fluid.

The commonest viral infections during HIV are caused by Herpes Simplex Virus, Cytomegalovirus, Varicella Zoster Virus. CMV causes retinitis and pneumonitis. HSV and VZV infections can be treated by acyclovir.

OPPORTUNISTIC TUMOURS

The common tumour is Kaposi's sarcoma which is vascular in nature is seen in 20% of patients with AIDS. In an average, 35% of patients have diseases of the mucosal membrane of the oral cavity, larynx, and most commonly the oesophagus, stomach and the intestines are affected. Excision and local management with vincristine are the palliative treatment.

Lymphomas of the HIV-infected individuals differ from other known lymphoma individuals by their site, degree of malignancy, and response to therapy. HIV-associated lymphomas are often found outside the lymphatic system, mostly in the brain, GI tract, bone marrow, and skin. Their response to treatment is less compared to that of classical lymphomas.

NEUROLOGICAL MANIFESTATIONS

Neurological changes present in HIV-infected patients ranges from mild neuropsychological symptoms such as memory loss, mood disturbances and behavior abnormalities to organic psychosis and dementia. The most common neurological disorder is subacute encephalitis (AIDS dementia complex and AIDS encephalopathy) which is present in 33% of cases. Other manifestations are peripheral neuropathy, aseptic meningitis, and acute meningoencephalitis.

DERMATOLOGICAL MANIFESTATIONS

Skin manifestations are seen in all stages of HIV infection which can be the first manifestation which makes the patient to give medical attention. Oral hairy leukoplakia, Seborrhoeic eczema and pruritis with maculopapular eruption are frequently observed in AIDS. Acne and allergic skin eruptions are also common. Skin eruptions such as

molluscum contagiosum, herpes zoster, condylomata acuminatum are also frequently present.

GASTROINTESTINAL MANIFESTATIONS

Persistent diarrhoea which is profuse is a recurrent problem in AIDS patients. Shigella, Salmonella, Giardia lamblia, Entamoeba histolytica, and Campylobacter are responsible for the persistent diarrhea. Other organisms such as Cryptosporadium, Mycobacterium avium or Karposi's sarcoma may also present with persistent diarrhea in patients with AIDS.

COURSE OF HIV INFECTION:

Typical progressors

Rapid progressors

Longterm – nonprogressors (LTNP s)

Typical progressors :

Typical progressors constitute an average of 85% of HIV infected individuals with a survival time of about 10 years. It has three phase's namely primary infection, clinical latency and clinically apparent disease. Progression of HIV to full blown AIDS occurs in period of 8-10 years approximately.

Rapid progressors:

Rapid progressors constitute about 10% of HIV infected individuals with a median survival time of about 3-4 years after seroconversion. Progression of HIV is rapid in these individuals because of defective immune response and CD8 cell mediated suppression.

Longterm-nonprogressors(LTNP s):

About 5% of the HIV infected individuals are called Longterm – non progressors (LTNPs). These individuals have stable CD4 cell count without any therapy for a long period. The CD4 count in these individuals

is usually more than 500. They have a high titre of neutralizing antibodies to HIV. Cell mediated immune response and humoral mediated immune responses are high in these persons. Viral load and replication of virus is four fold higher in these individuals in the lymph node and blood.[61]

REPLICATION CYCLE OF HIV:

The HIV life cycle includes the series of events that starts from the attachment of virus to the CD4 receptors on the host cell surface, and ends with the release of new viruses that bud off from the new host cell. HIV can infect several cells in our body, but its target is the CD4 lymphocyte. When a CD4 lymphocytic cell is infected with HIV, it undergoes many steps to replicate and generate many new viruses.

The replication of HIV can occur inside the human cells only. The replication process begins when a virus particle enter into a cell that carries on its surface a special protein called CD4. The virus particle contains spikes on its surface which stick to the CD4 and permit the viral envelope to fuse with the cell membrane. The HIV genomic particle then gets released into the cell, and leaves the envelope on the surface of the cell.[66]

Binding and Fusion:

The surface membrane of living cells consists of complex protein structures called receptors. The HIV life cycle begins when the HIV binds to the CD4 receptor and its co-receptors on the membrane surface of a CD4 T-lymphocyte. Then it fuses with the host cell, thereby releasing RNA into the host cell. The firm attachment of the viral particle to the CD4 receptor helps in fusion of HIV with the cell membrane and release of its genome into the host cell.

Reverse Transcription:

The enzyme called reverse transcriptase which reads the sequence of viral RNA nucleic acid and converts the single – stranded HIV RNA to double-stranded HIV DNA.

Integration:

After the reverse transcription of viral RNA, the HIV DNA enters the nucleus of the host cell; the enzyme integrase hides the HIV DNA. The integrated HIV DNA is called provirus which becomes inactive for many years, making copies of HIV[66].

Transcription:

The transcription occurs only when the lymphocyte is activated. After receiving a signal, the provirus utilizes the host enzyme RNA polymerase, to make copies of the HIV RNA, and also shorter strands of RNA named messenger RNA. The mRNA acts as a blueprint to create long chains of HIV proteins.

Assembly:

The Protease enzyme splits up the long chain of HIV proteins into small proteins. When the smaller HIV proteins and the HIV genomic material come together, a new virus particle is formed.

Budding:

The virus which is formed newly will be pushed out of the host cell which is called as budding. The new formed virus takes a portion of the cell's outer envelope which is covered with protein and sugar combinations called HIV glycoproteins. These glycoproteins are essential for the virus to bind CD4 and co receptors. The new HIV copies can infect other cells.

HIV replication is related with a high mutation rate because reverse transcription does not permit for rectification of errors in nucleotide incorporation. HIV evolves million times faster than the human genome

evolution thereby making tough for the immune system to identify and efficiently combat.[67]

LABORATORY DIAGNOSIS OF HIV:

HIV diagnostic testing has become available for the detection of antibodies to HIV only in the 1980s. To detect antibody as early as possible after infection, the enzyme immunoassays are sensitive. To confirm positive antibody screens, a variety of other assays are available.

Purpose of HIV testing[69]

- For prophylaxis and management.
- For blood safety and donation safety.
- For monitoring the epidemic (sentinel surveillance)
- Identification of asymptomatic individuals.
- To encourage for behavior modification through counselling.
- To diagnose clinically suspected cases.
- Voluntary testing after counseling

SEROLOGICAL TEST INCLUDES:

a) Antibody tests
b) Antigen tests

(a) Antibody tests:

For screening of blood samples for antibody to HIV Enzyme Linked Immunosorbent Assay (ELISA) is the most commonly performed method. The sensitivity and specificity of the present systems reaches 100% but false positive and false negative results cannot be excluded. Other antibody tests are Western blots, immunofluorescence, passive particle agglutination, and RIPA bioassays. Western blots are considered as the gold standard and seropositivity is diagnosed when antibodies against both the env and the gag proteins are detected[69].

(b) Antigen tests:

Before the appearance of antibody to HIV; antigen can be detected early in the course of HIV infection. Antigen is not detectable during the latent period of HIV infection but become it becomes detectable during the last stages of the infection.

Demonstration of viral nucleic acid:

This can be accomplished by probes or by PCR techniques. The Polymerase chain reaction may be exceptionally useful because of its extremely high sensitivity.

Methods to detect specific HIV antibodies:

i. Screening tests

ii. Supplemental tests

SCREENING TESTS:

The Enzyme-Linked Immunosorbent Assay (ELISA) usually takes 2-3 hours to yield the results. Rapid screening tests give results within minutes and include visual assays like dot blot tests, particles (gelatin, RBC, latex, micro beads) agglutination, HIV spot and comb test and fluorometric micro particle technologies. These tests are easy to perform, rapid, do not require sophisticated equipment, technical expertise and are mostly cost-effective. They also distinguish HIV-1 and HIV-2 antibodies.[70]

ELISA

The Enzyme-Linked Immunosorbent Assay is the most commonly performed screening test which is easy to carry out for a large sample size. It is highly sensitive, specific and cost-effective. The Enzyme-Linked Immunosorbent Assay is a technique used to detect the presence of an antigen/antibody in a specimen sample. It utilizes two antibodies, one is specific to the antigen and the other is coupled to an enzyme. The second antibody produces a fluorogenic or chromogenic substrate to

produce a signal. It is a device for determining serum antibody as well as the antigen. ELISA based on indirect method is used in most of the kits.[70]

SUPPLEMENTAL TESTS:

The samples which are reactive in the screening tests were confirmed with the help of supplemental tests for the confirmation of diagnosis.

When the sample is reactive by any one of the screening tests, it is tested by another different system to confirm the diagnosis. If a specimen is reactive in 2 different systems, it has to be tested again using one of the supplemental tests which may be a third ELISA Enzyme-Linked Immunosorbent Assay or Rapid test or a Western Blot test (WB).

WESTERN BLOT

For detecting HIV antibodies, the Western blot is the most commonly performing confirmatory assay. It is considered as the gold standard for confirmation of HIV infection. This technique is based on electrophoresis which denatures the proteins of the viral components and imparts negative charge to the antigens, and separates the antigens on the basis of their molecular weight. The separation of antigens permits the identification of specific antibodies to the viral antigens.

RAPID ASSAYS:

- Particle agglutination
- Dotblot assays
- Immunochromatography
- HIV spot and comb tests
- Dipstick and comb assays

There are many rapid assays which work on the principle of agglutination and Enzyme-Linked Immunosorbent Assay has been developed for quick results and easy performance. These rapid assays usually require 30 minutes or lesser time for performing the test and does not require any special equipment's.[70]

DOT BLOT ASSAYS

This rapid assay is easy to perform, and it can generally distinguish between HIV-1 and HIV-2 and do not require complicated equipments. The results are indicated by development of color. One of the drawbacks of the test is the high cost. Sensitivity and specificity of these assays are comparable with ELISA. These assays are beneficial for single test application (e.g: autopsy room and blood banks, emergency).

The immune chromatographic assays are 1-step rapid assay test kit, which has tools for screening, consisting of a flat cartridge device, plastic

or paper. Blood, serum or other body fluid is placed at the tip of the device and are allowed to diffuse along a strip that is impregnated with chemicals mostly colloidal gold that bind and permit visual detection of HIV antibodies some use third-generation. These tests can be completed in less than 10 minutes and even less than two minutes, no need for addition of reagents, and has control reagent to reduce technical errors. These can be stored at a different temperature and are transported simply without any consequences.

Based on type of HIV antigen, various types of HIV kits are available. First generation kits utilises antigens derived from viruses grown in human lymphocytes. Second generation kits utilise artificially derived recombinant antigens from fungi or bacteria. Third generation kits use chemically manufactured oligopeptides.

Laboratory diagnosis during window period:

Window period is a period of seronegativity during which an infected person do not give a positive result to either Western blot or the Enzyme-Linked Immunosorbent Assay, even though the viral load is high. These individuals may show certain symptoms and this period can last for 6 months before seroconversion. .

During window period HIV infection can be detected by viral demonstration and viral components by:

- Polymerase Chain Reaction
- p 24 antigen test
- Culture of virus[69]
- Nucleic acid amplification test (NAT) which detects viral nucleic acid.

Other laboratory findings for HIV infection may include leukopenia, anaemia and thrombocytopenia. Increased ESR, increased blood gammaglobulin, and decreased blood cholesterol level. Post exposure testing is suggested at six weeks, three months, and then after six months.

The important target of HIV replication is CD4 cell which results in depletion of CD4 cells. Therefore CD4 cell count is a marker for staging the HIV infection and monitoring the disease progression .The rate of HIV replication is also reflected by increase in plasma viral RNA load and is considered as one of the most specific and sensitive test to monitor the HIV progression

VIRAL LOAD TEST:

Viral load test is defined as the number of copies of HIV in 1ml of blood specimen. The viral load tests determines the amount of HIV RNA in a small quantity of blood .The viral load test is valuable for managing therapy response, evaluation of newly diagnosed HIV infection, surveillance of patients not receiving drug therapy. It will help to predict how long the patient will stay healthy.

TREATMENT

There is no cure for AIDS. The target of antiretroviral treatment is to bring the viral load in the body to a low level by suppressing the replication of the HIV virus in the body. This helps to reduce the weakening of the immune system and allows it to recover from damage that HIV might have caused previously. Combination of three or more than three anti-HIV drugs is referred to as Highly Active Antiretroviral Therapy (HAART). The most common drug combination comprises of two nucleoside reverse transcriptase inhibitor with either a non-nucleoside reverse transcriptase inhibitor or a protease inhibitor[71].

Highly Active Antiretroviral Therapy (HAART) has improved life expectancy and prognosis in patients with HIV infection. There has been 80% decrease in mortality after its introduction. Sometimes there may be resistant to one combination of Highly Active Antiretroviral Therapy (HAART), especially in patients who skip their medications.

Antiretroviral drugs in general fall into the following groups[72]:

- Nucleoside analogues – Inhibits the reverse transcriptase enzyme thereby preventing replication
- Non-nucleoside analogues – Inhibits reverse transcriptase enzyme.

- Protease inhibitors – Prevent viral protein processing.

- Fusion inhibitor – Inhibit the fusion of viral and cellular membranes.

Lifestyle factors such as exercise, diet, and stopping smoking, and recreational drug use are also important in HIV management.

POST EXPOSURE PROPHYLAXIS OF HIV:

Post-exposure prophylaxis refers to the management to reduce the risk of infection following exposure to blood-borne infectious organisms. At present there are only two ways to diminish the risk of developing HIV infection following exposure to HIV post-exposure prophylaxis (PEP) and interventions to prevent transmission from mother-to-child.[74]

Post-exposure prophylaxis (PEP) comprises taking anti-HIV drugs as soon as possible following exposure to HIV to decrease the chance of becoming HIV positive. The aim of post-exposure prophylaxis (PEP) is to decrease or prevent local viral replication prior to spread of infection, so that the infection can be abandoned.

There are two categories of post-exposure prophylaxis (PEP):

(1) Occupational PEP, (“oPEP”)

(2) Non-occupational PEP, (“nPEP”)

Occupational exposure (oPEP) is said to have occurred when a person working in a medical setup is exposed to materials which is infected with HIV. nPEP is when a person is exposed to HIV in some other situations like (condom breakage, sexual assault, etc.) To be effective, PEP must begin within 72 hours of exposure, before the viral replication. PEP consists of 2-3 antiretroviral medications and should be taken for 28 days. In addition counseling should be provided because of the psychosocial issues.[74]

POST EXPOSURE PROPHYLAXIS REGIMEN[73]:

Basic two drug regimen (For low risk)

Zidovudine 300 mg + Lamivudine 150 mg - Twice daily for 4 weeks

Expanded three drug regimen (For high risk)

Zidovudine 300 mg + Lamivudine 150 mg - Twice daily for 4 weeks

+ Indinavir 800 mg (or another PI) - Thrice daily for 4 weeks

LOW RISK:

- When the source of infection is positive for HIV, but asymptomatic with low HIV-RNA titre and high CD4 cell count.
- When the exposure is through the mucous membrane, or superficial scratch, or through thin and solid needle.

HIGH RISK:

- When the source is symptomatic AIDS patient with high HIV - RNA titre or low CD4 count.
- Exposure is through major splash or large area contact of longer duration with mucous membrane or abraded skin or through small gauge needle, puncture wound which is deep, and patient's blood on the needle.

UNIVERSAL PRECAUTIONS DURING AUTOPSY[83]:

Universal precautions are intended to follow for blood, vaginal secretions and semen as well as to peritoneal, pericardial cerebrospinal, synovial, pleura fluids, and it is believed that tears, nasal secretions, sweat, sputum, urine, faeces and vomitus and amniotic fluids are not infectious unless they contain visible blood.

Only experts and health care professionals who are skilled in handling the infected material are allowed to enter in to the postmortem examination room. Only these experienced professionals should conduct the autopsy examination because it is proved that the risk of accidental infection occurs more common in inexperienced persons. Studies reported that laceration occurred in 1 in every 11 autopsies conducted by inexperienced persons. Individuals with immune deficiency and persons

who have open wounds or skin lesions like dermatitis should not involve in postmortem examination.

The postmortem examination room should be sufficient without overcrowding and proper ventilation should be provided. Protective measures such as gloves, headwear, masks, eyewear, shoes and full-covered gown should be used by the mortuary staff while conducting the postmortem examination. Gloves should be checked for leakage frequently for cuts and puncture. Glove perforation occurs commonly, double gloving in the dissection room is suggested to reduce the risks of cutaneous blood contacts.

Hand washing is mandatory after handling blood and body fluids contamination and also after the removal of gloves. Contamination with blood and/or other body fluids must not be moved from gloved hands to other surfaces which may subsequently be touched by ungloved hands like door handles, telephone receiver, table, chair, etc. No hand-to-hand passing of Sharp instruments while performing postmortem examination and it should be handled with great care. Disposable syringes, needles and other sharps should be retained in puncture resistant containers for their safe disposal.

PREVENTION OF PERINATAL TRANSMISSION OF HIV

The rate of HIV transmission can be reduced to two-thirds by administration of zidovudine to the women during pregnancy, labor, and delivery and also to their newborns. Zidovudine treatment is more effective when it is administered during labour or administered to the infant, if the treatment is begun in less than 48 hours after birth.[74]

If zidovudine resistance is suspected, combination antiretroviral treatment is recommended. A regimen of oral zidovudine between 14 and 34 weeks of gestation (100 mg five times a day), intravenous zidovudine during labor, and zidovudine syrup from birth through 6 weeks of age has been shown to reduce the rate of vertical (mother-to-newborn) transmission of HIV by up to 23%.

PREVENTION:

There is no effective vaccine against HIV. The only way to prevent infection is to avoid certain risk factors, such as sharing needles or unprotected sex.

Primary Prevention

Factors that determine the prevention of HIV infection:

- Precautions concerning safe sexual act and safe injection,

- HIV prophylaxis in perinatal period,
- Blood products screening,
- Infection control in medical profession.

Secondary Prevention

The disease progression can be significantly reduced with the presently available effective treatment. Along the current effective treatment, available prophylactic regimens to opportunistic infections prevent the infection and improve the survival rate of the HIV patients.

MATERIAL AND METHODS

The present study was conducted at the Institute of Forensic Medicine, Chennai-3. The study sample consists of 486 routine autopsy cases at Rajiv Gandhi Government General Hospital. Before getting into the study, Ethical clearance was obtained from the Ethical committee.

Blood samples were collected from a sum of 486 cases autopsied at Rajiv Gandhi Government General Hospital. The samples were collected via cardiac chamber or femoral vessel at the time of autopsy. The samples were tested blindly that the identity of the individual was unknown. None of the cases had a previous or known HIV status. The positive results were confirmed by another rapid assay test.

The samples were tested using an enzyme immunoassay using SD Bioline HIV1/2 3.0 Rapid test kits to detect the presence of HIV-1 and HIV-2 antibodies. The test procedure was performed according to the protocols supplied by the manufacturers. Samples yielding reactive results were confirmed by Alere Determine^{TM} HIV 1/2 method which detects HIV-1, HIV-2 antibodies. The present study was carried out for a period of 1 year on a dead bodies submitted for medicolegal autopsies at the institute of Forensic Medicine, Chennai-3.

The tested samples are anonymous, and it is collected without informed consent. Samples are anonymous, if it is impossible under any situation for anyone to identify the source of the sample. It is an epidemiological testing for the HIV prevalence in a particular population with the minimum of participation bias.

The individual's data such as demographics, cause of death, postmortem interval and positivity for the type of HIV (HIV-1 and/orHIV-2) are recorded. SD BIOLINE HIV1/2 3.0 Rapid test kit is an immunochromatographic test for the detecting antibodies to all isotypes (IgM, IgG) specific to HIV-1 and HIV-2 concurrently in human serum, plasma or whole blood qualitatively.

SD BIOLINE HIV1/2 3.0 SPECIFICATIONS:

The SD BIOLINE HIV1/2 3.0 Rapid test has a membrane strip which has two test band regions. Test band 1 has precoated recombinant HIV 1 capture antigen (gp24, p41). Test band 2 has precoated recombinant HIV2 capture antigen (gp36). The recombinant HIV1/2 antigen (gp24, p41 and gp36) gold conjugate and the sample move chromatograpically to the test region (T) and develop a visible line as the antigen – antibody-antigen gold particle complex with a high degree of specificity and sensitivity. This test device has a letter of 1 for test Line 1

(HIV-1), letter 2 for test line 2(HIV-2) and C representing control line respectively on the surface. Test lines and control line are not visible before applying any sample. The control line is signifies the procedural control. Control should appear if test procedure is performed properly.

PROCEDURE

After opening the foil pouch the test device is placed on a dry, clean, bright, flat surface. With the help of capillary pipette 20µl of blood specimen which is taken directly from the cardiac vessel or femoral vessel puncture is added into the sample well (It is indicated with the letter 'S') in the test device, then four drops of assay diluent is added in to the sample well .If the test is in progress, the purple colour moves across the result window which is in the center of the test device. Interpretation of the test results was done within 5-20 minutes.

INTERPRETATION

A colour band that appears in the left section of the test device signifies that the test is properly working. This band is control line (C).

Colour bands that appear in the right and middle section of the result window are the test lines. The bands are test line 1(1) and test line 2 (2).

NEGATIVE RESULT

The presence of only control line (C) in the test device designates a negative result.

POSITIVE RESULT

The presence of two lines - control line(C) and test line 1 (1) within the test device designates a positive result for HIV 1. The presence of two lines as control line(C) and test line 2 (2) within the test device designates a positive result for HIV 2. The occurrence of three lines as control line(C) and test line 1 (1) and test line 2 (2) within the test device designates a positive result for both HIV-1and HIV-2.

INVALID RESULT

Absence of control line (C) within the result window specifies an invalid result. The instructions are followed incorrectly or the test sample may have been deteriorated. Therefore the specimen should be re-tested.

LIMITATIONS OF THE TEST

To diagnose AIDS, Immunochromatographic testing alone cannot be used even if the antibodies against HIV-1 and/or HIV-2 are present in a deceased specimen. At the same time, negative result does not exclude

the possibility of HIV-1 and/or HIV-2 infection. This is because the specimen may contain low levels of antibodies to HIV-1 and /or HIV-2.

PERFORMANCE CHARECTERISTICS

Sensitivity – 100%Specificity – 99.8%

SPECIFICATIONS OF ALERE DETERMINE™ HIV 1/2

The Alere Determine™ HIV-1/2 is an in vitro, qualitative immunoassay which is visually read for the detection of HIV-1 and HIV-2 antibodies in human serum, whole blood or plasma.

PRINCIPLES:

Alere Determine™ HIV-1/2 is an immunochromatographic test for the detection of antibodies to HIV-1 and HIV-2 qualitatively. When a sample is added to the sample pad, it migrates through the conjugate pad; it mixes with the selenium colloid-antigen conjugate. This migrates through the solid phase to the immobilized recombinant antigens and synthetic peptides at the window site.

If HIV-1 and/or HIV-2 antibodies are present in the sample, the antibodies bind to the antigen as well as to the antigen-selenium colloid at the patient window, forming a red line there.

If HIV-1 and/or HIV-2 antibodies are absent in the sample, the red line will not be formed as the antigen-selenium colloid flows past the patient window. A control bar is incorporated in the assay device to insure assay validity.

TEST PROCEDURE

The protected foil covering the test card was removed. 50 µl of sample was applied to the sample pad, followed by a drop of chase buffer after 1 minute. The results were read after a minimum interval of 15 min.

LIMITATIONS OF THE TEST

1. The Alere Determine^{TM} HIV-1/2 detects HIV-1 and HIV-2 antibodies in whole blood, serum, plasma. Other body fluids or pooled specimens may not give accurate results.
2. The intensity of the patient bar does not necessarily correlate to the titre of antibody in the specimen.
3. A negative result will not ignore the possibility of HIV infection.

False negative result occur if,

- Low Antibody levels
- Infection with a variant of the virus
- Specimen handling conditions which result in loss of HIV antibody multivalency.

4. Whole blood or plasma specimen containing anticoagulants other than EDTA may give incorrect results.

PERFORMANCE CHARECTERISTICS

Sensitivity and specificity- 100%

ANALYSIS AND RESULTS

Of the 486 samples tested, HIV antibodies were detected in 4 samples using SD BIOLINE HIV1/2 3.0 Rapid test kit. The 4 positive samples were tested again by another rapid assay Alere Determine™, which were also positive for HIV-1. Data such as demographics, cause of death, postmortem interval and positivity for HIV-1 and or HIV-2 are recorded.

Out of the 4 positive samples, all were positive for HIV 1and none were positive for HIV 2, all were male and all the four positive cases were not previously known to have HIV-infection. The presence of two murder cases in the four HIV positives indicates that HIV positive cases are clustered in homicide cases.

Males occupy predominant number of cases, accounts for about 85.8 % of study sample, whereas female constitute only 14.2 % of the study sample. Among the positive samples all were male, none of the samples were positive for female indicating that seroprevalence is higher in males (100%).

Table 1: Sex distributions among the study sample:

Sex	No. of sample	No. of positive cases	Percentage of positive cases
Male	417	4	0.719
Female	69	0	0
Total	486	4	0.823

Fig 1: Sex distributions among the study sample:

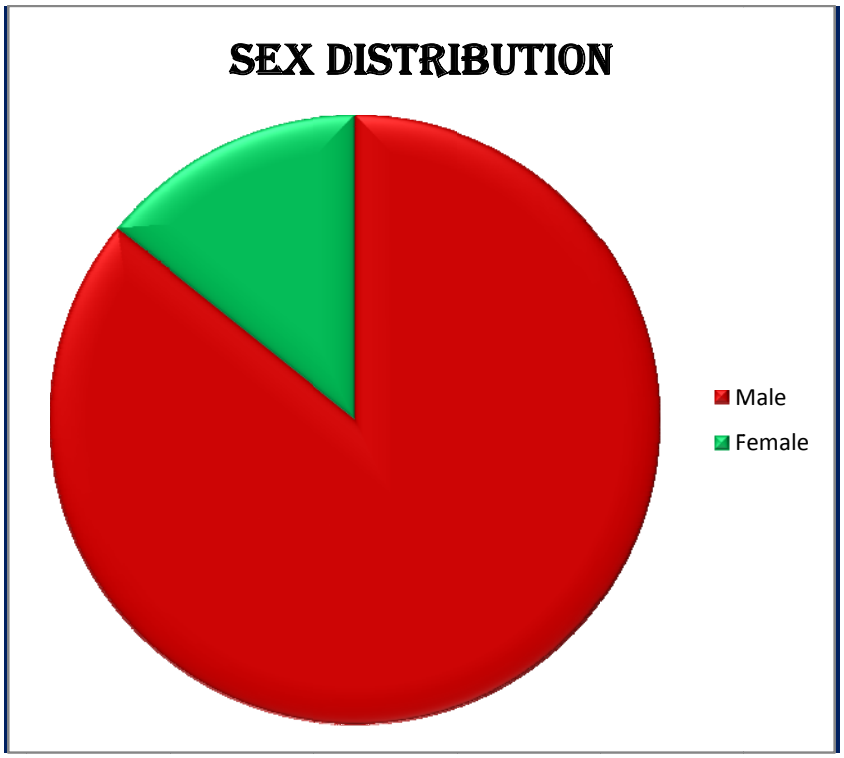
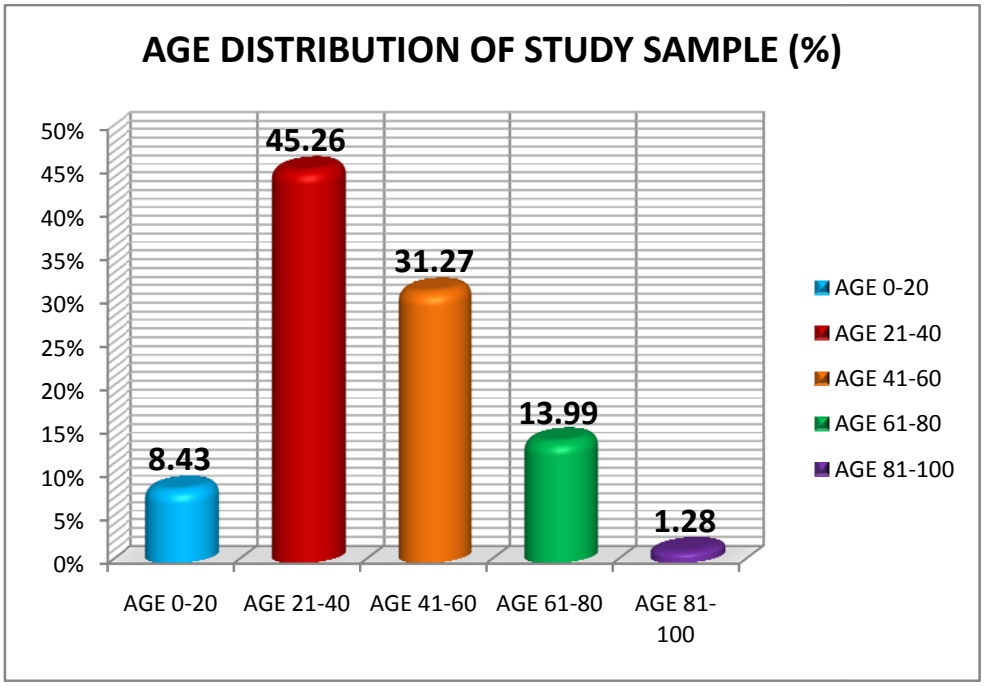


Table 2: Age distribution among of positive cases of the study sample

S.no	Age distribution	No. of sample	No. of positive cases
1.	Age 0-20 years	41	0
2.	Age 21-40 years	220	1
3.	Age 41-60 years	152	1
4.	Age 61-80 years	68	2
5.	Age 81-100 years	5	0
6.	Total	486	4

Fig 2. Age distribution of the study sample:



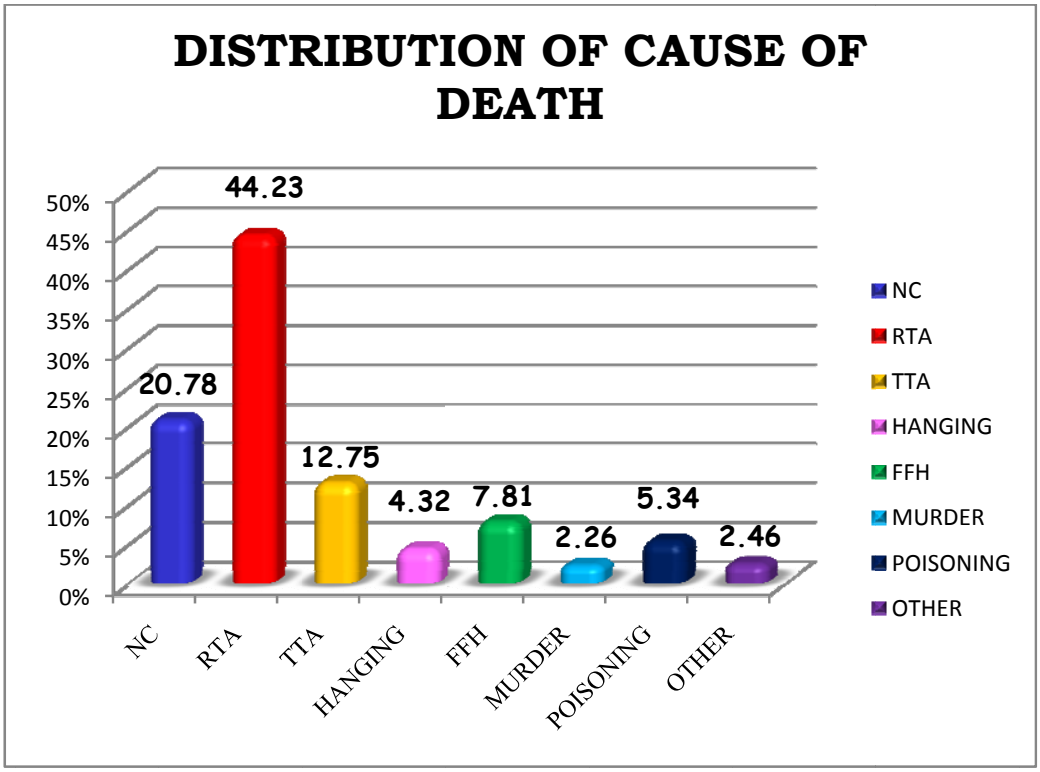
Among 486 cases analyzed, predominant number of cases falls under age group between 21 and 40, followed by age group between 41 and 60.In this study, Out of the four cases two cases were positive in the age group 61-80, indicating that highest seroprevalence was in the age group 61-80.

Table 3: Distribution of cause of death among the positive cases of the study sample:

S.no	Cause of death	No. of sample	No. of positive cases	Percentage of positive cases
1.	Natural cause	101	1	0.99
2.	Road traffic accident	215	0	0
3.	Train traffic accident	62	1	1.61
4.	Hanging	21	0	0
5.	Fall from height	38	0	0
6.	Murder	11	2	18.1
7.	Poisoning	26	0	0
8.	Other causes*	12	0	0
9.	Total	486	4	0.82

* (Snake bite, Drowning, Electrocution, Burns)

Fig 3. Distribution of cause of death among the study sample:



When looked in to the cause of death in the positive cases, the two cases out of four were homicide (50%). Out of the total eleven murder cases in this study two were positive (18.1%). This shows that HIV screening is of great importance among the community where the crime rate is high. Homicide victims in our study showed a relatively higher prevalence of HIV-1, infections compared with other manners of deaths.

DISCUSSION

The HIV estimation in India was first done in 1994 based on data from different sites. The People Living with HIV/AIDS (PLHA) in India in the year 2007 is estimated to be 2.31 million. Globally there are about 33.4 million people living with AIDS (PLHA) in the year 2008. HIV prevalence in adults as per the year 2009 estimate was 0.31%, which was higher among males than females.

A simple, reliable, and rapid test to detect HIV infection can be useful in the mortuary because it is not possible always to get the complete and correct information about all of the risk factors before commencing the postmortem, because of the social and cultural restrictions. Therefore the lack of a known history of such a risk factor does not equate with the nonexistence of such risk factor. Hence testing for HIV in medico-legal autopsies will identify carriers in whom HIV status was not previously known.

In countries like Iran, Finland, Germany etc., screening of HIV antibodies in autopsy was done as a back-up surveillance programs based on voluntary tests[75]. In the population where refusals are common for voluntary HIV tests, screening during postmortem efficiently reveals the true incidence of HIV without bias. Among 7305 medico-legal autopsies tested from 1986 to 1990 at Finland, nine cases were positive for HIV

(0.12%). This percentage is greater than the prevalence of 0.01 to 0.03% in voluntary screening programs from the general population. In this study, the four positive cases out of 486 cases were not known to have HIV which means they are clinically undetected for HIV antibodies. This indicates that that people at high risk are clustered in the medico-legal autopsy series[76].

There are many studies that have estimated the survival of the HIV postmortem. Viable HIV can be isolated from blood at autopsy up to 21 hours of postmortem interval under situations of proper body storage[76]. Nyberg et al isolated viable HIV up to 14 days postmortem. Karhunen et al. suggested from his results that HIV antibodies can be detected in biological fluids, if stored for months even at room temperature[75]. During the early phase of infection, the viral load in blood and other body fluids may be very high. This suggests the need for efficient protection of autopsy personnel's during all autopsies.

The testing of postmortem sera for antibodies to HIV will be a most reliable and accurate measure of antemortem HIV infection. Antibodies to HIV have been shown to remain detectable in postmortem serum stored for relatively long periods of time, more than 60 days at 4°C and up to 176 days at -70°C without degradation[77]. Furthermore, it has been demonstrated that extensive hemolysis of cadaveric blood does not

interfere with ELISA or Western blot analysis of HIV seropositivity. In general, proteins such as the globulins that comprise antibodies may not be affected by postmortem decomposition, hemolysis, or bacterial contamination[77,18]. In this study, it was estimated that the postmortem interval was ranging from 8 hours to 26 hours for detection of HIV antibodies.

Postmortem viral level is influenced by several factors like viral burden of death, viral strain, pre-mortem antiviral therapy and temperature maintained in the mortuary. Blood, vaginal fluid, semen, breast milk, and cerebrospinal, amniotic, pericardial and synovial fluids are the body fluids responsible for transmitting the HIV. Others such as saliva, tears, urine are not implicated in the transmission of HIV except they contain adequate blood.[19,42]

British clinical laboratories conducted a study between 1970 and 1989 and established that the highest rate of laboratory- acquired infections was in mortuary workers. Weston and Lober have recognized that about 8% surgical gloves get punctured during post-mortem and nearly one third of that was undetected by the pathologist, thus causing the infectious blood to be bathed in any preexisting hand injuries for a prolonged period of time.[25]

In the present study, the samples were tested blindly, that the identity of the individual was unknown. This study includes all types of forensic autopsy cases representing the general population. Samples of blood were collected from 486 autopsy cases at Rajiv Gandhi Government General Hospital. All the samples were tested for antibodies to both HIV-1 and HIV-2 using rapid standard ELISA kits which has a sensitivity of 100% and specificity of 99.8%.

Out of 486 cases, there are four positive cases and all the positive cases were male. These positive cases were further confirmed with another rapid-assay kit for HIV antibodies which is also simple, rapid and no special equipment is required and even whole blood can be used. This rapid-assay kit has a very high sensitivity of 100% and specificity of 100% in trials conducted in Asia and tests both HIV-1 and HIV-2. The presence of 4 positive cases among 486 cases, though statistically insignificant is higher than many other voluntary screening programs. In many voluntary screening programs there are people who abstain from testing, which does not affect in this study. Thus, screening in medico legal autopsy is a sensitive indicator epidemiologically.

Many studies conducted for detecting the antibodies to HIV in the autopsy population were classified the deceased as “known risk” and “no known risk” groups based on the history such as infection, drug

addiction, and sexual contact but that was not reliable and leads to misclassification. So, in this study such data about the risk factors were not collected.

There are two opinions concerning HIV in autopsies. One school of thought maintains that all autopsies should be carried with universal precaution which is almost impracticable in a developing country[79,80]. The second thought recommends pre autopsy testing for HIV by rapid test kits in the mortuary by taking blood samples from the dead body. If the result is positive, then universal precautions to be observed while conducting autopsies.[8,83]

LIMITATION OF THE STUDY:

The obstacles for postmortem testing of blood and body fluids include hemolysis, autolysis, bacterial contamination, and loss from decomposition. These obstacles are enhanced by prolonged postmortem intervals. However, immunoglobulins, are considered less likely to be affected by decomposition, hemolysis and bacterial contamination[79,18].

This screening test for the antibodies to HIV may not be sensitive if someone infected shortly before death and not seroconverted.

CONCLUSION

The present study concludes that testing of HIV in medico legal autopsies is a convenient and effective method in monitoring the surveillance of HIV-infection in the general population and it can be used for epidemiological studies. It could be used along with unlinked anonymous tests from hospital and other similar patient materials.

Testing for HIV may also be desired for safety reasons in mortuaries[81]. In screening postmortem blood for HIV-antibodies, the present study represents that rapid assay test pack is simple, rapid, no special equipment are required, even whole blood can be used and has very high sensitivity of 100% and specificity of 99.8% and tests both HIV 1 and HIV 2.

Out of 486 subjects in this study group, 4 cases were positive for HIV and all were previously unknown seropositive cases. Though the rate of infection appears to be less than 1%, even with a needle stick, it is not practical or economical to take universal precautions with every autopsy. It would, therefore, be advantageous to know a deceased HIV serological status prior to autopsy. Screening may be worthwhile in cases at high risk of HIV infection where the HIV antibody status is not known at the time of postmortem.

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MASTER CHART

S.NO	P.M.NO	SEX	AGE	CAUSE OF DEATH	HIV-1	HIV-2	CONFIRMATORY TEST
1	1418/12	M	50	RTA	Negative	Negative	Negative
2	1419/12	M	40	RTA	Negative	Negative	Negative
3	1420/12	M	70	RTA	Negative	Negative	Negative
4	1421/12	F	45	NC	Negative	Negative	Negative
5	1422/12	M	70	NC	Negative	Negative	Negative
6	1423/12	M	18	RTA	Negative	Negative	Negative
7	1424/12	M	38	NC	Negative	Negative	Negative
8	1425/12	M	45	NC	Negative	Negative	Negative
9	1426/12	M	38	RTA	Negative	Negative	Negative
10	1427/12	F	30	RTA	Negative	Negative	Negative
11	1428/12	M	26	RTA	Negative	Negative	Negative
12	1429/12	F	53	RTA	Negative	Negative	Negative
13	1430/12	M	31	RTA	Negative	Negative	Negative
14	1431/12	M	40	Fall	Negative	Negative	Negative
15	1432/12	M	27	Hanging	Negative	Negative	Negative
16	1433/12	M	80	NC	Negative	Negative	Negative
17	1434/12	M	32	RTA	Negative	Negative	Negative
18	1435/12	M	55	NC	Negative	Negative	Negative
19	1436/12	M	63	NC	Negative	Negative	Negative
20	1437/12	M	24	RTA	Negative	Negative	Negative
21	1438/12	M	52	Hanging	Negative	Negative	Negative
22	1439/12	F	83	TTA	Negative	Negative	Negative
23	1440/12	M	49	NC	Negative	Negative	Negative
24	1441/12	M	59	RTA	Negative	Negative	Negative
25	1442/12	M	42	Fall	Negative	Negative	Negative
26	1443/12	M	78	Drowning	Negative	Negative	Negative

27	1444/12	M	18	RTA	Negative	Negative	Negative
28	1445/12	M	35	RTA	Negative	Negative	Negative
29	1446/12	M	29	Fall	Negative	Negative	Negative
30	1447/12	M	32	RTA	Negative	Negative	Negative
31	1448/12	M	42	NC	Negative	Negative	Negative
32	1449/12	M	86	RTA	Negative	Negative	Negative
33	1450/12	M	45	NC	Negative	Negative	Negative
34	1451/12	M	70	NC	Negative	Negative	Negative
35	1452/12	M	55	NC	Negative	Negative	Negative
36	1453/12	M	62	Fall	Negative	Negative	Negative
37	1454/12	M	30	RTA	Negative	Negative	Negative
38	1455/12	M	60	NC	Negative	Negative	Negative
39	1456/12	M	35	RTA	Negative	Negative	Negative
40	1457/12	M	23	RTA	Negative	Negative	Negative
41	1458/12	M	28	RTA	Negative	Negative	Negative
42	1459/12	M	62	RTA	Negative	Negative	Negative
43	1460/12	M	45	RTA	Negative	Negative	Negative
44	1461/12	M	30	Fall	Negative	Negative	Negative
45	1462/12	M	25	RTA	Negative	Negative	Negative
46	1463/12	F	27	Poisoning	Negative	Negative	Negative
47	1464/12	F	52	RTA	Negative	Negative	Negative
48	1465/12	M	26	Hanging	Negative	Negative	Negative
49	1466/12	M	60	RTA	Negative	Negative	Negative
50	1467/12	M	60	NC	Negative	Negative	Negative
51	1468/12	M	24	Poisoning	Negative	Negative	Negative
52	1469/12	M	50	TTA	Negative	Negative	Negative
53	1470/12	M	32	RTA	Negative	Negative	Negative
54	1471/12	F	2	Poisoning	Negative	Negative	Negative
55	1472/12	M	60	NC	Negative	Negative	Negative

56	1473/12	M	54	RTA	Negative	Negative	Negative
57	1474/12	M	40	RTA	Negative	Negative	Negative
58	1475/12	F	38	RTA	Negative	Negative	Negative
59	1476/12	M	37	RTA	Negative	Negative	Negative
60	1477/12	M	28	RTA	Negative	Negative	Negative
61	1478/12	M	30	TTA	Negative	Negative	Negative
62	1479/12	F	60	NC	Negative	Negative	Negative
63	1480/12	M	33	Fall	Negative	Negative	Negative
64	1481/12	M	25	RTA	Negative	Negative	Negative
65	1482/12	F	25	NC	Negative	Negative	Negative
66	1483/12	M	55	Hanging	Negative	Negative	Negative
67	1484/12	M	65	NC	Negative	Negative	Negative
68	1485/12	M	35	NC	Negative	Negative	Negative
69	1486/12	M	45	NC	Negative	Negative	Negative
70	1487/12	M	45	Burns	Negative	Negative	Negative
71	1488/12	M	70	NC	Negative	Negative	Negative
72	1489/12	M	55	NC	Negative	Negative	Negative
73	1490/12	M	65	NC	Negative	Negative	Negative
74	1491/12	M	70	TTA	Negative	Negative	Negative
75	1492/12	F	42	Poisoning	Negative	Negative	Negative
76	1493/12	M	30	NC	Negative	Negative	Negative
77	1494/12	M	65	NC	Negative	Negative	Negative
78	1495/12	M	25	TTA	Negative	Negative	Negative
79	1496/12	M		NC	Negative	Negative	Negative
80	1497/12	M	59	NC	Negative	Negative	Negative
81	1498/12	F	9	TTA	Negative	Negative	Negative
82	1500/12	M	35	Hanging	Negative	Negative	Negative
83	1501/12	M	68	TTA	Negative	Negative	Negative
84	1502/12	M	27	RTA	Negative	Negative	Negative

85	1503/12	F	38	RTA	Negative	Negative	Negative
86	1504/12	M	34	RTA	Negative	Negative	Negative
87	1505/12	F	30	RTA	Negative	Negative	Negative
88	1506/12	M	70	Fall	Negative	Negative	Negative
89	1507/12	M	80	NC	Negative	Negative	Negative
90	1508/12	M	28	TTA	Negative	Negative	Negative
91	1509/12	M	27	NC	Negative	Negative	Negative
92	1510/12	M	21	RTA	Negative	Negative	Negative
93	1511/12	M	76	RTA	Negative	Negative	Negative
94	1512/12	M	22	RTA	Negative	Negative	Negative
95	1513/12	M	72	RTA	Negative	Negative	Negative
96	1514/12	M	27	RTA	Negative	Negative	Negative
97	1515/12	M	27	Hanging	Negative	Negative	Negative
98	1516/12	M	14	Hanging	Negative	Negative	Negative
99	1517/12	M	37	Fall	Negative	Negative	Negative
100	1518/12	M	27	RTA	Negative	Negative	Negative
101	1519/12	M	65	NC	Negative	Negative	Negative
102	1520/12	M	18	RTA	Negative	Negative	Negative
103	1521/12	M	68	NC	Negative	Negative	Negative
104	1522/12	M	33	RTA	Negative	Negative	Negative
105	1523/12	M	32	Fall	Negative	Negative	Negative
106	1524/12	M	55	Fall	Negative	Negative	Negative
107	1525/12	M	32	Poisoning	Negative	Negative	Negative
108	1526/12	F	28	Poisoning	Negative	Negative	Negative
109	1527/12	F	24	Hanging	Negative	Negative	Negative
110	1528/12	M	25	Fall	Negative	Negative	Negative
111	1529/12	F	28	Hanging	Negative	Negative	Negative
112	1530/12	M	38	RTA	Negative	Negative	Negative
113	1531/12	F	26	Poisoning	Negative	Negative	Negative

114	1532/12	M	10	RTA	Negative	Negative	Negative
115	1533/12	M	48	RTA	Negative	Negative	Negative
116	1534/12	M	60	Murder	Positive	Negative	Positive
117	1535/12	M	50	RTA	Negative	Negative	Negative
118	1536/12	M	25	Fall	Negative	Negative	Negative
119	1537/12	M	83	TTA	Negative	Negative	Negative
120	1538/12	M	40	RTA	Negative	Negative	Negative
121	1539/12	M	40	RTA	Negative	Negative	Negative
122	1540/12	M	18	RTA	Negative	Negative	Negative
123	1541/12	M	70	NC	Negative	Negative	Negative
124	1542/12	M	45	Murder	Negative	Negative	Negative
125	1543/12	M	42	NC	Negative	Negative	Negative
126	1544/12	M	39	Poisoning	Negative	Negative	Negative
127	1545/12	M	38	Poisoning	Negative	Negative	Negative
128	1546/12	M	26	RTA	Negative	Negative	Negative
129	1547/12	M	17	TTA	Negative	Negative	Negative
130	1548/12	F	33	Poisoning	Negative	Negative	Negative
131	1549/12	F	23	NC	Negative	Negative	Negative
132	1550/12	F	56	Snake bite	Negative	Negative	Negative
133	1551/12	F	48	Snake bite	Negative	Negative	Negative
134	1552/12	M	19	TTA	Negative	Negative	Negative
135	1553/12	M	29	RTA	Negative	Negative	Negative
136	1554/12	M	25	Fall	Negative	Negative	Negative
137	1555/12	M	48	RTA	Negative	Negative	Negative
138	1556/12	M	31	RTA	Negative	Negative	Negative
139	1558/12	M	27	TTA	Negative	Negative	Negative
140	1559/12	M	35	RTA	Negative	Negative	Negative
141	1560/12	M	66	RTA	Negative	Negative	Negative
142	1561/12	M	60	RTA	Negative	Negative	Negative

143	1562/12	F	28	NC	Negative	Negative	Negative
144	1565/12	M	23	TTA	Negative	Negative	Negative
145	1567/12	M	42	RTA	Negative	Negative	Negative
146	1568/12	M	48	RTA	Negative	Negative	Negative
147	1571/12	M	18	TTA	Negative	Negative	Negative
148	1572/12	M	43	TTA	Negative	Negative	Negative
149	1573/12	M	38	Fall	Negative	Negative	Negative
150	1574/12	M	43	TTA	Negative	Negative	Negative
151	1575/12	M	48	RTA	Negative	Negative	Negative
152	1576/12	M	51	RTA	Negative	Negative	Negative
153	1577/12	M	22	RTA	Negative	Negative	Negative
154	1579/12	M	26	Fall	Negative	Negative	Negative
155	1580/12	M	55	RTA	Negative	Negative	Negative
156	1581/12	M	40	NC	Negative	Negative	Negative
157	1582/12	M	55	RTA	Negative	Negative	Negative
158	1583/12	F	21	RTA	Negative	Negative	Negative
159	1584/12	F	60	Poisoning	Negative	Negative	Negative
160	1585/12	M	22	RTA	Negative	Negative	Negative
161	1587/12	M	41	NC	Negative	Negative	Negative
162	1588/12	M	26	NC	Negative	Negative	Negative
163	1589/12	M	30	RTA	Negative	Negative	Negative
164	1590/12	F	40	RTA	Negative	Negative	Negative
165	1592/12	M	43	Poisoning	Negative	Negative	Negative
166	1593/12	M	35	Hanging	Negative	Negative	Negative
167	1594/12	F	24	RTA	Negative	Negative	Negative
168	1595/12	F	2 Month	RTA	Negative	Negative	Negative
169	1597/12	M	57	RTA	Negative	Negative	Negative
170	1598/12	F	26	RTA	Negative	Negative	Negative

171	1599/12	M	35	TTA	Negative	Negative	Negative
172	1602/12	M	45	RTA	Negative	Negative	Negative
173	1605/12	M	24	RTA	Negative	Negative	Negative
174	1606/12	M	36	NC	Negative	Negative	Negative
175	1607/12	M	35	NC	Negative	Negative	Negative
176	1609/12	M	75	NC	Negative	Negative	Negative
177	1611/12	M	23	Poisoning	Negative	Negative	Negative
178	1616/12	M	52	RTA	Negative	Negative	Negative
179	1619/12	M	50	RTA	Negative	Negative	Negative
180	1620/12	M	20	Fall	Negative	Negative	Negative
181	1621/12	M	54	NC	Negative	Negative	Negative
182	1622/12	M	44	RTA	Negative	Negative	Negative
183	1626/12	M	55	RTA	Negative	Negative	Negative
184	1629/12	M	29	TTA	Negative	Negative	Negative
185	1630/12	F	35	RTA	Negative	Negative	Negative
186	1632/12	M	65	NC	Negative	Negative	Negative
187	1633/12	M	19	TTA	Negative	Negative	Negative
188	1634/12	M	27	RTA	Negative	Negative	Negative
189	1635/12	M	45	NC	Negative	Negative	Negative
190	1636/12	M	52	TTA	Negative	Negative	Negative
191	1640/12	M	57	NC	Negative	Negative	Negative
192	1647/12	F	35	RTA	Negative	Negative	Negative
193	1648/12	M	23	RTA	Negative	Negative	Negative
194	1653/12	M	31	Hanging	Negative	Negative	Negative
195	1654/12	M	72	RTA	Negative	Negative	Negative
196	1655/12	M	47	RTA	Negative	Negative	Negative
197	1656/12	M	22	TTA	Negative	Negative	Negative
198	1658/12	M	62	Murder	Negative	Negative	Negative
199	1659/12	M	32	TTA	Negative	Negative	Negative

200	1663/12	M	28	RTA	Negative	Negative	Negative
201	1664/12	M	65	Poisoning	Negative	Negative	Negative
202	1666/12	M	45	RTA	Negative	Negative	Negative
203	1667/12	M	45	RTA	Negative	Negative	Negative
204	1668/12	M	72	RTA	Negative	Negative	Negative
205	1669/12	M	47	TTA	Negative	Negative	Negative
206	1673/12	M	60	Fall	Negative	Negative	Negative
207	1674/12	M	42	TTA	Negative	Negative	Negative
208	1675/12	M	50	Fall	Negative	Negative	Negative
209	1678/12	M	41	RTA	Negative	Negative	Negative
210	1679/12	M	27	RTA	Negative	Negative	Negative
211	1681/12	M	41	NC	Negative	Negative	Negative
212	1682/12	M	54	RTA	Negative	Negative	Negative
213	1683/12	F	35	RTA	Negative	Negative	Negative
214	1686/12	F	18	Hanging	Negative	Negative	Negative
215	1687/12	M	17	Hanging	Negative	Negative	Negative
216	1689/12	M	18	RTA	Negative	Negative	Negative
217	1690/12	M	41	TTA	Negative	Negative	Negative
218	1694/12	F	22	RTA	Negative	Negative	Negative
219	1695/12	M	42	TTA	Negative	Negative	Negative
220	1696/12	M	68	NC	Negative	Negative	Negative
221	1698/12	M	59	RTA	Negative	Negative	Negative
222	1700/12	M	26	RTA	Negative	Negative	Negative
223	1701/12	F	25	Poisoning	Negative	Negative	Negative
224	1703/12	M	53	TTA	Negative	Negative	Negative
225	1705/12	M	36	Fall	Negative	Negative	Negative
226	1707/12	M	35	RTA	Negative	Negative	Negative
227	1715/12	M	5	Burns	Negative	Negative	Negative
228	1726/12	M	32	Snake bite	Negative	Negative	Negative

229	1727/12	M	26	TTA	Negative	Negative	Negative
230	1728/12	M	39	RTA	Negative	Negative	Negative
231	1731/12	M	22	RTA	Negative	Negative	Negative
232	1732/12	M	30	RTA	Negative	Negative	Negative
233	1733/12	M	17	TTA	Negative	Negative	Negative
234	1735/12	F	50	RTA	Negative	Negative	Negative
235	1741/12	F	30	RTA	Negative	Negative	Negative
236	1745/12	M	40	Murder	Positive	Negative	Positive
237	1746/12	F	65	RTA	Negative	Negative	Negative
238	1747/12	M	35	RTA	Negative	Negative	Negative
239	1752/12	M	19	TTA	Negative	Negative	Negative
240	1753/12	M	50	Fall	Negative	Negative	Negative
241	1754/12	M	24	RTA	Negative	Negative	Negative
242	1755/12	F	27	Hanging	Negative	Negative	Negative
243	1756/12	F	65	NC	Negative	Negative	Negative
244	1758/12	M	47	TTA	Negative	Negative	Negative
245	1759/12	M	49	RTA	Negative	Negative	Negative
246	1762/12	M	30	RTA	Negative	Negative	Negative
247	1763/12	M	55	RTA	Negative	Negative	Negative
248	1767/12	F	78	RTA	Negative	Negative	Negative
249	1769/12	M	25	NC	Negative	Negative	Negative
250	1770/12	M	26	Murder	Negative	Negative	Negative
251	1771/12	M	42	RTA	Negative	Negative	Negative
252	1772/12	M	13	RTA	Negative	Negative	Negative
253	1775/12	M	23	RTA	Negative	Negative	Negative
254	1777/12	M	80	RTA	Negative	Negative	Negative
255	1778/12	F	17	TTA	Negative	Negative	Negative
256	1779/12	F	25	Poisoning	Negative	Negative	Negative
257	1780/12	M	65	RTA	Negative	Negative	Negative

258	1781/12	M	21	RTA	Negative	Negative	Negative
259	1782/12	M	21	RTA	Negative	Negative	Negative
260	1783/12	M	64	RTA	Negative	Negative	Negative
261	1784/12	M	24	RTA	Negative	Negative	Negative
262	1788/12	M	31	RTA	Negative	Negative	Negative
263	1790/12	M	25	Fall	Negative	Negative	Negative
264	1792/12	M	41	RTA	Negative	Negative	Negative
265	1793/12	F	70	RTA	Negative	Negative	Negative
266	1795/12	M	14	RTA	Negative	Negative	Negative
267	1797/12	M	45	Poisoning	Negative	Negative	Negative
268	1800/12	M	26	TTA	Negative	Negative	Negative
269	1801/12	M	24	Murder	Negative	Negative	Negative
270	1802/12	M	50	RTA	Negative	Negative	Negative
271	1803/12	M	65	NC	Positive	Negative	Positive
272	1804/12	M	22	NC	Negative	Negative	Negative
273	1805/12	M	34	Hanging	Negative	Negative	Negative
274	1806/12	M	48	Fall	Negative	Negative	Negative
275	1807/12	M	60	NC	Negative	Negative	Negative
276	1808/12	M	25	Hanging	Negative	Negative	Negative
277	1809/12	M	20	TTA	Negative	Negative	Negative
278	1810/12	M	40	RTA	Negative	Negative	Negative
279	1811/12	F	65	RTA	Negative	Negative	Negative
280	1812/12	F	55	Murder	Negative	Negative	Negative
281	1813/12	M	33	RTA	Negative	Negative	Negative
282	1814/12	M	24	Hanging	Negative	Negative	Negative
283	1815/12	M	27	Poisoning	Negative	Negative	Negative
284	1816/12	M	63	RTA	Negative	Negative	Negative
285	1817/12	M	35	RTA	Negative	Negative	Negative
286	1818/12	M	26	Fall	Negative	Negative	Negative

287	1819/12	F	75	Fall	Negative	Negative	Negative
288	1820/12	M	23	TTA	Negative	Negative	Negative
289	1821/12	F	67	TTA	Negative	Negative	Negative
290	1822/12	M	40	TTA	Negative	Negative	Negative
291	1823/12	M	50	TTA	Negative	Negative	Negative
292	1824/12	M	35	TTA	Negative	Negative	Negative
293	1825/12	M	27	TTA	Negative	Negative	Negative
294	1826/12	M	45	TTA	Negative	Negative	Negative
295	1827/12	M	50	NC	Negative	Negative	Negative
296	1828/12	M	36	TTA	Negative	Negative	Negative
297	1829/12	M	62	RTA	Negative	Negative	Negative
298	1830/12	M	72	RTA	Negative	Negative	Negative
299	1831/12	M	44	RTA	Negative	Negative	Negative
300	1832/12	M	65	Fall	Negative	Negative	Negative
301	1833/12	M	66	RTA	Negative	Negative	Negative
302	1834/12	M	55	RTA	Negative	Negative	Negative
303	1835/12	M	20	RTA	Negative	Negative	Negative
304	1836/12	M	30	RTA	Negative	Negative	Negative
305	1837/12	M	19	Fall	Negative	Negative	Negative
306	1838/12	M	70	NC	Negative	Negative	Negative
307	1839/12	M	60	RTA	Negative	Negative	Negative
308	1840/12	M	24	RTA	Negative	Negative	Negative
309	1841/12	M	35	TTA	Negative	Negative	Negative
310	1842/12	M	40	RTA	Negative	Negative	Negative
311	1843/12	M	31	RTA	Negative	Negative	Negative
312	1844/12	M	34	RTA	Negative	Negative	Negative
313	1845/12	M	70	RTA	Negative	Negative	Negative
314	1846/12	M	36	Poisoning	Negative	Negative	Negative
315	1847/12	M	33	NC	Negative	Negative	Negative

316	1848/12	M	60	NC	Negative	Negative	Negative
317	1849/12	M	70	RTA	Negative	Negative	Negative
318	1850/12	M	65	NC	Negative	Negative	Negative
319	1851/12	M	32	NC	Negative	Negative	Negative
320	1852/12	M	42	TTA	Negative	Negative	Negative
321	1853/12	M	20	RTA	Negative	Negative	Negative
322	1854/12	M	50	Fall	Negative	Negative	Negative
323	1855/12	M	93	TTA	Negative	Negative	Negative
324	1856/12	M	47	Poisoning	Negative	Negative	Negative
325	1857/12	M	20	RTA	Negative	Negative	Negative
326	1858/12	M	30	RTA	Negative	Negative	Negative
327	1859/12	M	50	TTA	Negative	Negative	Negative
328	1860/12	M	52	RTA	Negative	Negative	Negative
329	1861/12	F	20	Fall	Negative	Negative	Negative
330	1862/12	M	38	RTA	Negative	Negative	Negative
331	1863/12	M	23	Burns	Negative	Negative	Negative
332	1864/12	M	60	NC	Negative	Negative	Negative
333	1865/12	M	26	RTA	Negative	Negative	Negative
334	1866/12	M	24	RTA	Negative	Negative	Negative
335	1867/12	M	15	RTA	Negative	Negative	Negative
336	1868/12	F	53	RTA	Negative	Negative	Negative
337	1869/12	F	45	RTA	Negative	Negative	Negative
338	1870/12	M	58	NC	Negative	Negative	Negative
339	1871/12	M	35	Fall	Negative	Negative	Negative
340	1872/12	M	11	Fall	Negative	Negative	Negative
341	1873/12	M	43	Fall	Negative	Negative	Negative
342	1874/12	M	51	RTA	Negative	Negative	Negative
343	1875/12	M	39	RTA	Negative	Negative	Negative
344	1876/12	M	35	RTA	Negative	Negative	Negative

345	1877/12	M	43	TTA	Negative	Negative	Negative
346	1878/12	M	21	RTA	Negative	Negative	Negative
347	1879/12	M	45	NC	Negative	Negative	Negative
348	1880/12	M	75	NC	Negative	Negative	Negative
349	1881/12	M	59	NC	Negative	Negative	Negative
350	1882/12	M	26	Poisoning	Negative	Negative	Negative
351	1883/12	M	25	Hanging	Negative	Negative	Negative
352	1884/12	M	32	RTA	Negative	Negative	Negative
353	1885/12	F	37	RTA	Negative	Negative	Negative
354	1886/12	M	37	RTA	Negative	Negative	Negative
355	1887/12	M	76	NC	Negative	Negative	Negative
356	1888/12	M	35	TTA	Negative	Negative	Negative
357	1889/12	F	50	RTA	Negative	Negative	Negative
358	1890/12	M	45	NC	Negative	Negative	Negative
359	1891/12	M	45	NC	Negative	Negative	Negative
360	1892/12	M	20	TTA	Negative	Negative	Negative
361	1893/12	M	34	Murder	Negative	Negative	Negative
362	1894/12	M	62	NC	Negative	Negative	Negative
363	1895/12	M	29	Hanging	Negative	Negative	Negative
364	1896/12	M	42	RTA	Negative	Negative	Negative
365	1897/12	M	50	NC	Negative	Negative	Negative
366	1898/12	M	70	NC	Negative	Negative	Negative
367	1899/12	M	42	RTA	Negative	Negative	Negative
368	1900/12	M	31	Snake bite	Negative	Negative	Negative
369	1942/12	M	45	RTA	Negative	Negative	Negative
370	1973/12	M	21	RTA	Negative	Negative	Negative
371	1944/12	M	31	RTA	Negative	Negative	Negative
372	1943/12	M	60	RTA	Negative	Negative	Negative
373	2116/12	M	50	NC	Negative	Negative	Negative

374	2101/12	M	3	RTA	Negative	Negative	Negative
375	2974/12	M	17	Poisoning	Negative	Negative	Negative
376	2973/12	M	51	NC	Negative	Negative	Negative
377	2999/12	M	61	RTA	Negative	Negative	Negative
378	2406/12	F	25	TTA	Negative	Negative	Negative
379	2993/12	M	61	RTA	Negative	Negative	Negative
380	1918/12	M	50	NC	Negative	Negative	Negative
381	2046/12	F	80	NC	Negative	Negative	Negative
382	2833/12	F	60	NC	Negative	Negative	Negative
383	3012/12	F	65	Snake bite	Negative	Negative	Negative
384	2364/12	M	27	RTA	Negative	Negative	Negative
385	2023/12	M	50	Murder	Negative	Negative	Negative
386	2918/12	M	44	RTA	Negative	Negative	Negative
387	2453/12	F	50	Drowning	Negative	Negative	Negative
388	3011/12	M	42	RTA	Negative	Negative	Negative
389	3020/12	M	55	Poisoning	Negative	Negative	Negative
390	2834/12	F	55	NC	Negative	Negative	Negative
391	2232/12	M	33	Hanging	Negative	Negative	Negative
392	2141/12	M	35	Fall	Negative	Negative	Negative
393	3013/12	M	21	RTA	Negative	Negative	Negative
394	2889/12	M	20	RTA	Negative	Negative	Negative
395	2890/12	M	52	RTA	Negative	Negative	Negative
396	2913/12	M	65	RTA	Negative	Negative	Negative
397	2917/12	M	45	RTA	Negative	Negative	Negative
398	2975/12	M	25	RTA	Negative	Negative	Negative
399	2491/12	M	47	NC	Negative	Negative	Negative
400	2025/12	M	40	NC	Negative	Negative	Negative
401	2870/12	M	47	NC	Negative	Negative	Negative
402	2977/12	M	30	RTA	Negative	Negative	Negative

403	2844/12	M	50	NC	Negative	Negative	Negative
404	2366/12	M	38	RTA	Negative	Negative	Negative
405	2184/12	M	50	RTA	Negative	Negative	Negative
406	2077/12	M	40	RTA	Negative	Negative	Negative
407	2113/12	M	70	RTA	Negative	Negative	Negative
408	2137/12	F	35	NC	Negative	Negative	Negative
409	2405/12	M	70	NC	Negative	Negative	Negative
410	2215/12	M	18	RTA	Negative	Negative	Negative
411	2075/12	M	38	NC	Negative	Negative	Negative
412	2997/12	M	45	NC	Negative	Negative	Negative
413	2996/12	M	38	RTA	Negative	Negative	Negative
414	2216/12	F	30	RTA	Negative	Negative	Negative
415	2976/12	M	26	RTA	Negative	Negative	Negative
416	2234/12	F	53	RTA	Negative	Negative	Negative
417	2865/12	M	31	RTA	Negative	Negative	Negative
418	2401/12	M	40	Fall	Negative	Negative	Negative
419	2868/12	M	27	RTA	Negative	Negative	Negative
420	3016/12	M	80	NC	Negative	Negative	Negative
421	2966/12	M	32	RTA	Negative	Negative	Negative
422	2903/12	M	55	NC	Negative	Negative	Negative
423	2902/12	M	63	NC	Negative	Negative	Negative
424	2492/12	M	24	RTA	Negative	Negative	Negative
425	2117/12	M	52	Hanging	Negative	Negative	Negative
426	2406/12	F	83	TTA	Negative	Negative	Negative
427	2916/12	M	49	NC	Negative	Negative	Negative
428	288/12	M	59	RTA	Negative	Negative	Negative
429	2878/12	M	42	Fall	Negative	Negative	Negative
430	2453/12	M	78	Drowning	Negative	Negative	Negative
431	2190/12	M	18	RTA	Negative	Negative	Negative

432	2869/12	M	35	RTA	Negative	Negative	Negative
433	2965/12	M	29	Fall	Negative	Negative	Negative
434	2901/12	M	32	RTA	Negative	Negative	Negative
435	2835/12	M	42	NC	Negative	Negative	Negative
436	2871/12	M	86	RTA	Negative	Negative	Negative
437	2935/12	M	45	NC	Negative	Negative	Negative
438	2420/12	M	70	NC	Negative	Negative	Negative
439	2290/12	M	55	NC	Negative	Negative	Negative
440	2213/12	M	62	Fall	Negative	Negative	Negative
441	2235/12	M	30	RTA	Negative	Negative	Negative
442	2140/12	M	60	NC	Negative	Negative	Negative
443	2020/12	M	35	RTA	Negative	Negative	Negative
444	2169/12	M	23	RTA	Negative	Negative	Negative
445	2138/12	M	28	RTA	Negative	Negative	Negative
446	2165/12	M	62	RTA	Negative	Negative	Negative
447	2168/12	M	45	RTA	Negative	Negative	Negative
448	2292/12	M	30	Fall	Negative	Negative	Negative
449	2233/12	M	25	RTA	Negative	Negative	Negative
450	2970/12	F	27	Poisoning	Negative	Negative	Negative
451	2978/12	F	60	RTA	Negative	Negative	Negative
452	2400/12	M	26	Hanging	Negative	Negative	Negative
453	3000/12	M	60	RTA	Negative	Negative	Negative
454	3008/12	M	35	NC	Negative	Negative	Negative
455	3017/12	M	30	Poisoning	Negative	Negative	Negative
456	3019/12	M	50	TTA	Positive	Negative	Positive
457	2971/12	M	32	RTA	Negative	Negative	Negative
458	2979/12	F	2	Poisoning	Negative	Negative	Negative
459	3008/12	M	60	NC	Negative	Negative	Negative
460	2073/12	M	54	RTA	Negative	Negative	Negative

461	2210/12	M	40	RTA	Negative	Negative	Negative
462	2049/12	F	38	RTA	Negative	Negative	Negative
463	2090/12	M	37	RTA	Negative	Negative	Negative
464	2050/12	M	28	RTA	Negative	Negative	Negative
465	2021/12	M	24	TTA	Negative	Negative	Negative
466	1920/12	F	60	NC	Negative	Negative	Negative
467	2013/12	M	40	Fall	Negative	Negative	Negative
468	1945/12	M	25	RTA	Negative	Negative	Negative
469	2933/12	F	25	NC	Negative	Negative	Negative
470	2400/12	M	40	Hanging	Negative	Negative	Negative
471	2846/12	M	65	NC	Negative	Negative	Negative
472	2845/12	M	26	NC	Negative	Negative	Negative
473	2900/12	M	45	NC	Negative	Negative	Negative
474	1947/12	M	45	RTA	Negative	Negative	Negative
475	2915/12	M	70	NC	Negative	Negative	Negative
476	2052/12	M	55	NC	Negative	Negative	Negative
477	1919/12	M	65	NC	Negative	Negative	Negative
478	1956/12	M	70	TTA	Negative	Negative	Negative
479	2142/12	F	42	Poisoning	Negative	Negative	Negative
480	1922/12	M	30	NC	Negative	Negative	Negative
481	1925/12	M	65	NC	Negative	Negative	Negative
482	1914/12	M	30	TTA	Negative	Negative	Negative
483	1917/12	M	60	NC	Negative	Negative	Negative
484	1916/12	M	59	NC	Negative	Negative	Negative
485	1905/12	F	2	RTA	Negative	Negative	Negative
486	1975/12	M	72	RTA	Negative	Negative	Negative